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A Leader of the Movement for a Botanical Garden in the City of New York
Resulting in the Establishment of
The New York Botanical Garden
In Bronx Park, New York City
of which he was
Director-in-Chief, 1896–1929
Secretary of the Board of Managers, 1895–1929

To the Memory of This

DISTINGUISHED LEADER IN BOTANICAL SCIENCE

This Volume is
Affectionately Dedicated

The dedicatory page is contributed by John Hendley Barnhart, Bibliographer of the New York Botanical Garden and friend of long standing.

The portrait of Doctor Britton is reproduced from a photograph taken 22 June 1902 in the herbarium of the New York Botanical Garden by the staff photographer, Mr. F. Berte.

A recessive factor lethal for ascospore formation in Neurospora

B. O. Dodge

(with plates 9 and 10)

Ascus abortion in an x-rayed line of *Neurospora tetrasperma* was recently reported (Dodge, 1934) as due to a "lethal" which prevents the delimitation of ascospores and which segregates at meiosis. The lethal can not be maintained continuously in a haploid mycelium unless the nucleus which carries it is accompanied by a normal nucleus. Ascus abortion in the case described, therefore, occurs in an ascus heterozygous for the lethal. The present paper deals with a very different type of ascus abortion, an abortion which is due to a recessive gene, lethal in an ascus homozygous for this factor so that no ascospores are formed and the ascus sac itself finally disintegrates.

HOMOZYGOUS ASCUS ABORTION

Among the 1-ascospore cultures obtained by mating the mycelium from the x-rayed ascospore, G5.3, with normal race S1, was one (G5.3×S1) 9, that showed signs of reversion to normal. Testing out a number of 1-ascospore races from this mating, it was found that one race (G5.3×S1) 9.7, referred to hereafter simply as 9.7, developed perithecia very slowly at first, but later, it, as well as sub-cultures from it, matured perithecia much more rapidly. Although a great many fruit bodies, fairly normal in appearance, always develop in such cultures, and many asci reach full size in every case, the asci all abort, so that very few, if any, ascospores are ever formed (plate 9,C). The large hyaline highly refringent bodies in these aborting asci might be mistaken for spores. Lindegren (1934) unfortunately refers to some such bodies in an ascus of *N. crassa* as spores without nuclei. As will be shown cytologically, in our material they are merely vacuoles. In old cultures the perithecial cavity is filled with a rather formless mass of disintegration products. Sometimes the contents burst through the perithecial wall or through the ostiolar region to show as a whitish drop on the outside. Occasionally one finds a heavily indurated dark colored empty ascus, and more rarely a body that may represent an abnormal ascospore.

Since asci in perithecia of race 9.7 do not delimit spores, how can the race be bred? One can transfer to sub-cultures in the old way, but such transfers will be just like the original 9.7; they will, in fact, still be the original. There are, however, two different ways by which this race can be bred sexually so that its nature can be studied effectively. First, it can be

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grown in plate cultures opposite a normal unisexual race. The writer (Dodge, 1931) has shown that hermaphroditic races of *Neurospora* can be crossed or hybridized with unisexual races in this way. This method was recently tried out with some of the hermaphroditic races such as (G5.3×S1) 4.38 originating from the x-rayed series and which carry the first type of lethal for spore formation and ascus abortion (see plate 9,A). If the normal unisexual race opposed is of the sex opposite that of the normal nuclei in the hermaphroditic race, then two kinds of perithecia will be found. In addition to those containing indurated aborted asci produced on the hermaphroditic mycelium, there will be some normal perithecia without any sporeless indurated asci whatever. But if the unisexual race opposed is of the sex opposite to that of the lethal nuclei carried along in the hermaphroditic mycelium, then the perithecia are all alike and will all contain some aborted asci. On certain potato dextrose agars in tube cultures most of the asci abort, but on corn meal agar the lethal action is not complete so that ascospores sufficient for breeding purposes are produced, and it is not necessary to resort to this method of plate culturing.

In case of race 9.7 where, even under the most favorable conditions yet found, very few, if any, true ascospores are formed, the plate method proved valuable. When 9.7 was grown in a plate opposite normal unisexual races such as S1, sex B, and S6, sex A, perithecia with asci bearing spores were formed regardless of the sex of the normal race opposed. This proved that the 9.7 mycelium contains nuclei of opposite sex and suggested that these nuclei also carry something that prevents spore formation when two of them are united in an ascus; a nucleus of either kind, however, united with a normal nucleus of the opposite sex in an ascus becomes inoperative to produce abortion. Spores are then delimited. Since the mycelium is heterokaryotic sexually at least, perhaps something could be learned after all by studying its conidia to see how many kinds are formed and how mycelia derived from them would react in cultures of various sorts.

Conidial isolates from mycelium 9.7

Conidia from culture 9.7 were sowed in the usual way (Dodge, 1928b) and thirty-two 1-conidium isolates were obtained. In making the selections, the smaller and more slowly growing conidia were chosen in order to obtain unisexual mycelia. Twenty-three of the isolates proved to be hermaphroditic, each developing many perithecia, but in each case all of the asci aborted just as would be expected. The cultures were observed for the next four months without finding any changes except further degeneration.

The other nine isolates were unisexual. They were grown together in all possible combinations, and it was found that nos. 9.7C1, 9.7C4 and 9.7C5 were alike and of the same sex reaction, while 9.7C2, 9.7C3, 9.7C6, 9.7C7, 9.7C8 and 9.7C9 were of the opposite sex. The resulting perithecia contained great numbers of asci but no spores. Ascus abortion was complete. Each isolate was then in turn grown with the S1 tester, sex B, and the new S9 tester, sex A. Those of the first group proved to be sex A, and those of the second group sex B. The perithecia formed in these test cultures all bore asci with spores (plate 10,B).

It may be recalled that normal bisexual races of *N. tetrasperma* (Dodge, 1928b) produce three kinds of monilioid conidia. It has also been proved more recently that any bisexual race from the irradiated line G5.3, which produces the type of aborting asci shown in plate 9,A, also develops three kinds of monilioid conidia, but only two of the kinds are able to function vegetatively. Some are bisexual and their mycelia produce perithecia with the indurated aborted asci. Other conidia are unisexual and normal, that is, their nuclei do not carry the lethal; in any particular culture they are all of the same sex. Conidia carrying only lethal or deficient (?) nuclei are also abstricted, but they die soon after they germinate. In this respect they are just like the unisexual ascospores that carry only lethal nuclei.

Proof of a mutation or genetic change

Many cases of variations or saltations found in the mycelial and fruiting characters of the fungi have been referred to as mutations without any real evidence. Dixon (1933) shows some interesting effects of irradiating ascospores and mycelia of *Chaetomium*. His saltant mycelia in some cases produced numerous perithecia which matured spores, but the author did not prove the saltation to be genetic and hereditary. By testing out the ascospore progeny one could have seen whether the changes brought about by the irradiation were nuclear and hereditary and not merely cytoplasmic. For example, ascus abortion in case of our 9.7 race is not proved to be genetic even when mycelia obtained from its conidia are mated. On the contrary, it would at first seem to be merely cytoplasmic because ascospores in abundance mature when the conidial isolates are mated with normal tester races. Only when these f_1 ascospores are analyzed is it shown that the factors for this new type of ascus abortion are also segregated at meiosis and are hereditary.

Starting with culture 9.7C8×S9, fifty-six f_1 1-ascospore cultures were isolated. The small, and therefore unisexual, spores were selected where possible. Cultures nos. −1, −4, −9, −14, −53, −54, and −56, proved to be hermaphroditic and their perithecia mature asci with spores. The common

parental mating formula (9.7C8×S9) may be omitted. Nos. −3, −13, −19, −25, −27, −32, −33, −34, −42, −46, and −52 either died after germinating, or produced merely dwarf mycelia. These were eventually discarded without further test although they would have been interesting genetically. Nos. −7, −15, and −24 were lost accidentally. The other thirty-five races were mated against testers S1 and S9. Twenty-four proved to be of sex A and eleven sex B in their reaction. In all cases the resulting perithecia here also produced asci with spores. There were several cases of rather weak sexuality shown and some spore abortion but no case of complete lack of spores from ascus abortion such as characterizes race 9.7.

Back-crossing f_1 progeny with unisexual components of race 9.7

It usually requires from three to five days to get positive reactions. Ascospores begin to form about the sixth or seventh day. Some combinations fruit much more slowly. Each of the eleven sex B f_1 clones was mated against each of the three sex A conidial isolates from 9.7. The following sample matings, taken from the record, but omitting the details, illustrate the method of procedure. Only matings of clones of opposite sex are given here. The sign + means that perithecia were formed.

9.7C1×(9.7C8×S9) 5 = +, very strong reaction, many ascospores.
9.7C4×(9.7C8×S9) 5 = +, very strong reaction, many ascospores.
9.7C5×(9.7C8×S9) 5 = +, very strong reaction, many ascospores.
9.7C1×(9.7C8×S9) 8 = +, rather incompatible, complete ascus abortion.
9.7C4×(9.7C8×S9) 8 = +, very incompatible, complete ascus abortion.
9.7C5×(9.7C8×S9) 8 = −, completely incompatible in this culture.
9.7C5×(9.7C8×S9) 8 = +, weak sexuality, but complete ascus abortion.
9.7C1×(9.7C8×S9)12 = +, very strong, many ascospores.
9.7C4×(9.7C8×S9)12 = +, very strong reaction, many ascospores.
9.7C5×(9.7C8×S9)12 = +, very strong, many ascospores.
9.7C1×(9.7C8×S9)26 = +, very strong reaction, but complete ascus abortion.
9.7C4×(9.7C8×S9)26 = +, very strong, but complete ascus abortion.
9.7C5×(9.7C8×S9)26 = +, very strong, complete ascus abortion.
9.7C1×(9.7C8×S9)43 = +, very strong reaction, many ascospores.
9.7C4×(9.7C8×S9)43 = +, strong, many spores.
9.7C5×(9.7C8×S9)43 = +, very strong reaction, many spores.

These sample matings show that certain combinations resulted in the development of perithecia with asci but the asci aborted without delimiting spores. Other combinations gave what appeared to be perfectly normal perithecia with an abundance of spores. It is also evident that the three

conidial isolates 9.7C1, 9.7C4 and 9.7C5 are alike; they are the same clone in fact.

Each of the twenty-four f_1 sex A races was mated against the conidial isolate 9.7C2 which is sex B. The results were of the same nature. Some combinations gave normal perithecia with many ascospores, others produced fruit bodies with many asci but these all aborted without forming spores. Cases of weak sexual reactions were represented as before.

Judging from the results of the reactions there were four very definite classes of the f_1 ascospore progeny from the mating (9.7C8×S9). This was proved out further by mating or inbreeding the twelve sex B f_1 ascospore races in various combinations with the twenty-three sex A f_1 ascospore races so that each race was used at least once in some combination. A race derived from an ascospore is a definite clone and it is called here an ascospore race to distinguish it from a conidial isolate of 9.7. Four sample matings are given below.

(9.7C8×S9) 2×(9.7C8×S9) 8 = +, complete ascus abortion.

(9.7C8×S9) 6×(9.7C8×S9)10 = +, strong reaction, complete ascus abortion.

(9.7C8×S9) 6×(9.7C8×S9)22 = +, rather weak, but ascospores.

(9.7C8×S9) 6×(9.7C8×S9)44 = +, strong, many ascospores.

The results of a number of such matings in which every one of the thirty-five f_1 haplont clones was tested, proved conclusively that the factors responsible for ascus abortion are recessive, are segregated at meiosis and are therefore hereditary.

In order to further prove that the lethal carried by the conidial isolate clone 9.7C4, which is sex A, is the same as that carried by the isolate 9.7C8, sex B, a number of the f_1 progeny of the mating 9.7C4×S1 were also analyzed. A few of the results are given below. It should be held in mind that the thirty-five f_1 ascospores previously analyzed were from the mating 9.7C8×S9.

f_1 ascospore progeny of 9.7C4×S1 mating

From the mating 9.7C4×S1, fifty 1-ascospore f_1 clones were obtained in the usual way. Three germinating spores died after having been transferred. Eighteen mycelia turned out to be bisexual. The resulting perithecia all bore asci with spores. Twenty-nine of these f_1 clones were unisexual, thirteen being sex A and sixteen sex B.

Forty-one matings were then made, crossing the progeny of the mating 9.7C4×S1 with progeny of the mating 9.7C8S9. A few sample matings are given here to again indicate the method.

(9.7C8×S9)26×(9.7C4×S1) 4 = +, asci with spores.

(9.7C8×S9)18×(9.7C4×S1) 1=+, asci with spores.
(9.7C8×S9)26×(9.7C4×S1)16=+, with aborted asci only.
(9.7C8×S9) 6×(9.7C4×S1)39=+, with aborted asci only.
(9.7C8×S9)36×(9.7C4×S1)36=+, asci with ascospores.

Furthermore the f_1 progeny of the mating 9.7C4×S1 were inbred in twenty-five different combinations in the way the sample matings below indicate.

(9.7C4×S1) 8×(9.7C4×S1) 2=+, with aborted asci only.
(9.7C4×S1)14×(9.7C4×S1) 2=+, with aborted asci only.
(9.7C4×S1)16×(9.7C4×S1)46=+, asci with spores.
(9.7C4×S1)16×(9.7C4×S1)43=+, perithecia with aborted asci.

The results of all of these matings show that the f_1 progeny of the mating 9.7C4×S1 fall into four classes as do the progeny of the mating 9.7C8×S9. Whenever there is a mating between two clones each of which carries the same factor for ascus abortion, the ascus is homozygous for this factor, no spores are formed and ascus abortion is complete (plate 9, C). If one of the parents is normal, that is, non-lethal, and the other carries the factor for abortion, then the asci in the resulting perithecia are heterozygous for this factor and cut out spores as shown in plate 10, B.

For convenience merely, we have in the past designated the particular sex reactions of heterothallic clones of *Neurospora* by the letters A and B instead of by the signs + and −. As has been pointed out a number of times by the writer, normal or wild type races usually produce not only incipient perithecia in culture but also spermatia (microspores or microconidia). Both types of conidia function asexually in propagation, and both types function sexually as fertilizing elements. The fact that the spermatia will germinate to form perfectly normal mycelia and that the monilioid conidia serve beautifully as "spermatizers" (Dodge, 1932) has puzzled certain recent writers on this subject, and as such unorthodox behaviors do not fit into their categories they have ignored them altogether.

In the language of the geneticist it would certainly be much simpler to refer to the factors for sex reactions not as A and B, but as S and s, or some other pair of letters. Since we have always referred to our sex A and sex B tester strains as S9 and S1 respectively we shall in this instance use "A" and "a" to avoid confusion, "A" for the sex A factor and "a" for the sex B factor.[1] They are clearly fundamental factors governing the sex reactions and they are allelomorphic. We may also refer to the normal factor combination or condition whatever it may be, for delimitation of asco-

[1] See also Hans Zickler in Planta 22: 573–613, 1934, for a paper which has just come to hand too late for consideration here.

spores as L and the comparable inhibiting combination or gene as l, so that the four classes of clones discussed above may be designated by the symbols AL, aL, Al, and al. AL×aL gives normal ascospore production. Al×al results in complete ascus abortion. AL×al and Al×aL give ascospores. In a large population there should then be a three to one result: three combinations maturing asci with spores to one in which the asci abort completely without forming spores. The following shows the number in each class, grouping the progeny of the mating 9.7C4×S1 together and those of mating 9.7C8×S9 in a second group. The parental formula which should precede the number designating the particular f_1 clone is omitted in each case for the sake of brevity.

f_1 progeny of mating (9.7C4×S1)

AL: 1, 4, 25, 27, 30, 31, 35, 41, 44 (nine in all)
aL: 10, 12, 23, 36, 38, 40, 42, 45, 46, 50 (ten in all)
Al: 8, 14, 16, 48 (four in all)
al: 2, 3, 26, 34, 39, 43, (six in all)

f_1 progeny of mating (9.7C8×S9)

AL: 29, 30, 31, 37, 40, 41, 48, 49, 51, 55 (ten in all)
aL: 5, 12, 22, 23, 43, 44 (six in all)
Al: 2, 6, 16, 17, 20, 28, 35, 36, 38, 39, 45, 47, 50 (thirteen in all)
al: 8, 10, 11, 18, 21, 26 (six in all).

Summarizing, the mating tests show that: (1) any clone AL in either group, mated with any clone aL in either group, will give normal perithecia with asci containing normal spores; (2) any combination AL×al or any Al×aL mating will give perithecia with asci containing spores; and (3) any Al×al mating will give perithecia with all asci aborted. Due allowance should be made where, in a few cases, weak sex reactions come into play to slow down perithecium production. With sufficiently large populations the ratio of these matings that result in perithecia with ascospores to those resulting in complete ascus abortion should then be about 3:1.

One has only to recall the simplest case of albinism in maize to understand the genetics of this type of ascus abortion. Maize seedlings heterozygous for a recessive lethal for chlorophyll are green, but seedlings homozygous for the factor are white and soon die. Self-pollinated heterozygous plants give offspring in a 3:1 ratio, three green to one white.

LINKAGE

When grown on dextrose agar media the f_1 progeny classified above show some very striking differences in their cultural characteristics which

are not so apparent when they are grown on a corn meal agar. Some cultures develop a whitish more or less appressed aerial growth and the medium turns blackish at the surface. Other cultures develop an abundance of orange-colored conidia while the agar medium maintains its clear amber color for some time. The ratio of the number of the former to the number of the latter kind was 3:1. When the sex reaction and the presence or absence of the lethal factor, l, were considered it was seen that all of the clones showing the bright orange masses of conidia belong to the same particular group aL of the four classes as arranged above. The progeny of both matings, (9.7C4×S1) and (9.7C8×S9) showed the same differences.

As suggested to the writer personally by Dr. A. F. Blakeslee and again by Prof. E. W. Sinnott, this looks as though some factor or combination, say O, for orange-colored masses of conidia is inhibited by the same recessive lethal, l, which when homozygous also prevents ascospores formation. This factor O is linked with the sex reaction factor a, so that, introducing the symbols O and o, the four classes of clones would be indicated as follows: (Ao)L, (aO)L, (Ao)l, and (aO)l. The second class, (aO)L is the only kind that could produce the bright orange-colored masses of conidia because in the combination (aO)l the factor O is suppressed in its activities by the lethal factor l.

This would be in line with what has been pointed out before (Dodge, 1928a; 1928b) to the effect that the tester race S1 ("a") always seems to produce more of the orange-colored conidia than does race S6 ("A"). Our new tester S9 ("A") which was used in the present work is similar in this respect to S6. No proof that this character for conidia is linked has ever been offered however. In *Neurospora sitophila* albino non-conidial races such as 56.1 and 56.2 reacting as "a" were readily obtained (Dodge, 1930; 1931). Certain interspecific hybrids that would pass for *N. tetrasperma* were bred up so that one of the two unisexual components of a bisexual race was non-conidial and "a" in its sex reaction at the same time. Lindegren (1932) has suggested that there may be some linkage relations involving color character, the production of conidia, conditions for ascospore germination and sex reactions in the hybrids just referred to. Furthermore Lindegren (1933) has proved a linkage between the sex factor and "*pale*" in *Neurospora crassa*. Mr. F. L. Tai, who is working on this question in our laboratory, will publish his results in due time.

NUCLEAR BEHAVIOR IN ABORTING ASCI

Normally nuclei of *Neurospora tetrasperma* pair up after the third division in the ascus and cut out the spores, one nucleus of each sex going

to each spore. A fourth division occurs as the spores mature (Dodge, 1927). In case of those abnormal asci (plate 9, A) where abortion occurs in an ascus heterozygous for a lethal or deficiency, nuclear fusion occurs as usual, but the cytoplasm of the full grown ascus is apt to be very foamy and vacuolate (plate 10, G) and this disintegration may set in before the third division. The reduction divisions are certainly very abnormal and clear cut cases of the first, second, and third divisions are hard to make out. They are not always simultaneous and show many other irregularities. Several preparations show that the eight nuclei resulting from the third division may all divide again without cutting out any spores. Such asci invariably abort. The sixteen nuclei degenerate (plate 10, F, G), and the ascus sac begins to thicken and becomes dark colored. Occasionally the reduction divisions are sufficiently normal to allow for the delimitation of ascospores and we are thereby provided with a sufficient number for breeding purposes.

In case of those asci shown in plate 9, C and plate 10, A, where the abortion is due to a recessive factor that is effective to prevent ascospore formation only when in an ascus homozygous for this factor, preparations show that nuclear fusion and the three divisions occur rather normally. The eight resulting nuclei then move to the center of the ascus as though crowded into that position by the pressure of the enlarging vacuoles on either side (plate 10, C–E). The nuclei then all slowly degenerate. The nature of the hyaline bodies that look so much like spores in the asci (plate 9, C) is clear from the cytological preparations. They are merely vacuoles. Occasionally the eight degenerating nuclei are separated into two or three groups by intervening vacuoles (plate 10, C).

There is some evidence that the nuclei in these aborting asci may rarely undergo a fourth division before they degenerate. This may account for the few cases where one finds an aborted thickly indurated ascus among the colorless asci in the perithecia of race 9.7. Any temporary condition of the culture that would lead to a fourth precocious nuclear division may be all that is necessary to bring about the type of abortion where the ascus sac becomes indurated. This would not necessarily be a genetic change such as would be inherited like those illustrated in plate 9, A. Anything that would speed up the nuclear divisions so that a fourth division would occur before the spores could be cut out might prevent the normal working of the machinery concerned with spore delimitation. Or, to put it another way, there seems to be something in all of the species of *Neurospora* so far found that inhibits this fourth division until *after* the spores are formed, then it is allowed to take place. Suppress or remove the inhibitor and the differentiations that give the ascospores their wall characteristics are

applied, in the absence of ascospores, to the ascus sac itself which then becomes darkly colored, thick walled and striated much like mature giant ascospores. It would not be surprising to find that under certain conditions such asci if taken early enough would actually germinate! Sections show that they are quite devoid of nuclei and cytoplasmic contents after they have become fully indurated.

We have several species of ascomycetes, notably among the Ascobolaceae where the asci have more than eight spores, which means that there are regularly more than three nuclear divisions before spore formation. Some species have asci with sixteen spores, others have asci with thirty-two spores and so on up to *Thelebolus* where there must be ten successive nuclear division before the 1024 spores are cut out. This behavior is the normal procedure, however, for those species and particularly characterizes each one.

Ascus abortion of the type which ends with induration, then, can be genetic and heritable, or it can be a temporary and abnormal development not transmitted to the next generation, as reported previously (Dodge, 1934). The first type was evidently due to the effects of irradiating the parental ascospore. In the other cases mentioned at that time the abortion must have been due to some abnormal cultural condition which affected the fungus only locally and temporarily. Ascus abortion of the type described in the present paper is genetic and complete but it should be distinguished from cases of parthenocarpy where perithecial bodies without any asci are formed in cultures of race G5.3 when grown alone.

SUMMARY

Among the progeny derived from a mating of two mycelia, one of which had come from an x-rayed ascospore of *Neurospora tetrasperma*, was a bisexual clone 9.7 which develops perithecial fruit bodies containing numbers of asci, but these asci abort without forming ascospores. The mycelium of clone 9.7 develops three kinds of monilioid conidia. Some are bisexual and their mycelia will mature perithecia likewise with only aborting asci but no ascospores. The other conidia are unisexual. Mating their mycelia together in different combinations it was found that they were of two kinds sexually. Here the fertile matings gave perithecia with only aborted asci the same as clone 9.7 itself. Mating these unisexual clones with tester races S1 and S9 it was found that the fertile combinations always gave perithecia whose asci delimited ascospores. Analysis of these 1-ascospore clones proved that they were primarily of four sorts genetically, AL, aL, Al, and al, where A and a represent sex reaction or sterility factors and L and l represent factors governing the delimitation

of ascospores, l being recessive and lethal for spore formation in an ascus homozygous for it. The ratio of the number of matings that give perithecia with ascospores to the number of matings that give perithecia with only aborting asci theoretically should be about 3:1. In such an aborting ascus homozygous for l, nuclear fusion is followed by three successive nuclear divisions after which the eight nuclei move to the center of the ascus and slowly degenerate.

The f_1 ascospore clones when grown on certain dextrose media showed striking cultural differences in a ratio about 3:1. Three-fourths of the cultures or clones produced a rather limited amount of whitish, sometimes appressed, aerial growth with few conidia, and the medium was blackened. One-fourth of the clones produced bright masses of orange-colored conidia, and the medium maintained its clear amber color for some time. Such clones all fall in the group aL suggesting that there may be a factor which we may call "O" concerned with the type of growth, abundance of conidia and certain color effects, and which is linked with the sex reaction factor "a". This factor O is inhibited by the recessive factor "l" which is also lethal for ascospore formation when homozygous. In any series of the f_1 progeny one fourth, (aO)L, of the cultures will produce an abundance of bright orange-colored conidia while the other three fourths (Ao)L, (Ao)l, and (aO)l produce few conidia and a whitish more or less appressed aerial growth and the medium often becomes blackened.

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Explanation of plates 9 and 10

Neurospora tetrasperma

Plate 9

A. Reproduced from the writer's previous paper (1934) for comparison. Aborted asci are heterozygous for a lethal or deficiency resulting from irradiating a parental ascospore. Each of the spores in the 4-spored asci is provided at its origin with one nucleus carrying the lethal and one normal nucleus of opposite sex. In the 8-spored ascus, four spores are normal and four would carry only lethal nuclei and would die soon after germination.

B. Normal asci such as would develop when two mycelia derived from two of the normal spores of opposite sex shown in the 8-spored ascus in A are mated. The insert, lower right, shows a 5-spored ascus. The two small spores would be unisexual.

C. Aborted asci homozygous for a recessive factor, l, lethal for spore formation only when homozygous. The large hyaline spore-like bodies are merely vacuoles. Asci heterozygous for this factor delimit ascospores as shown in plate 10, B.

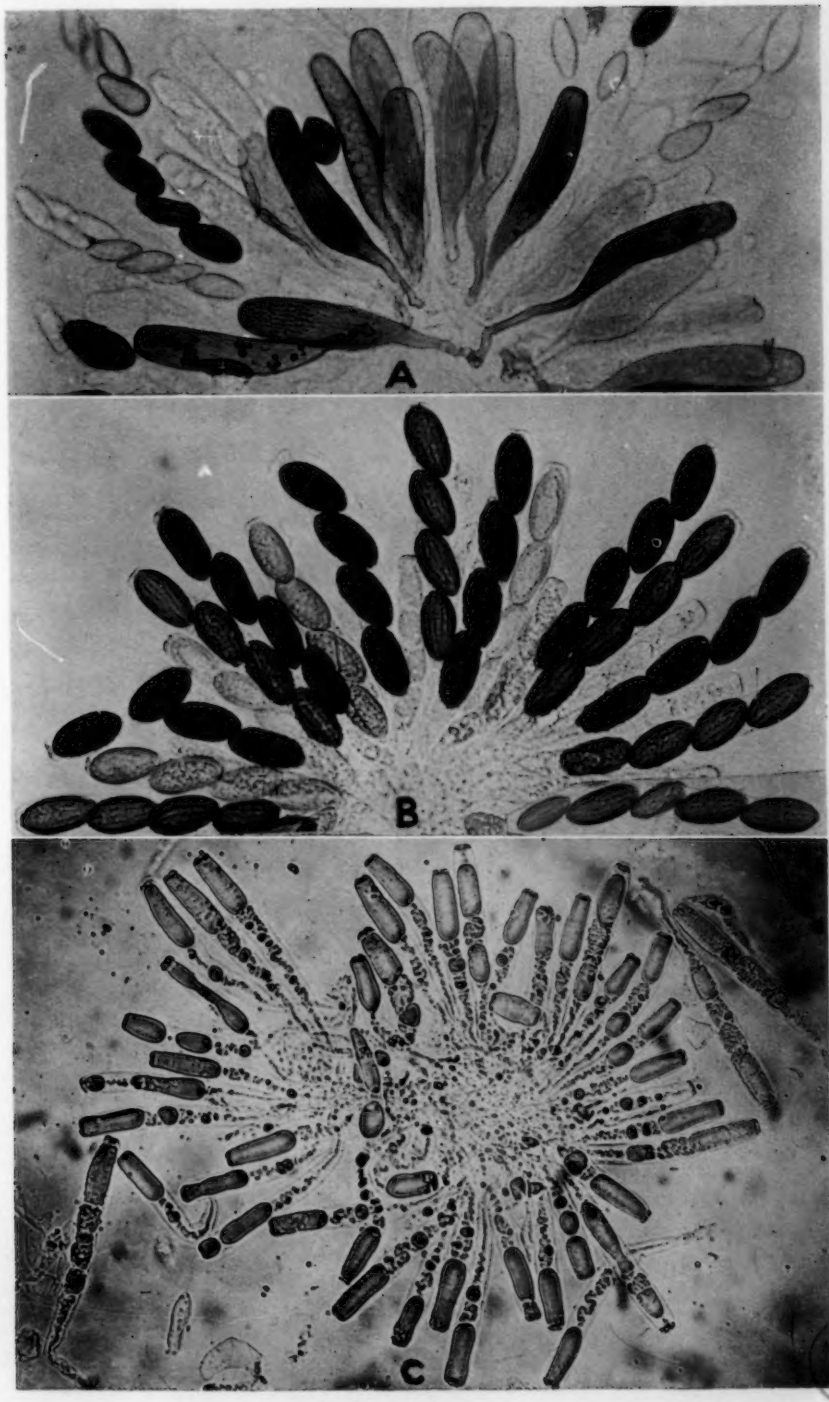
Plate 10

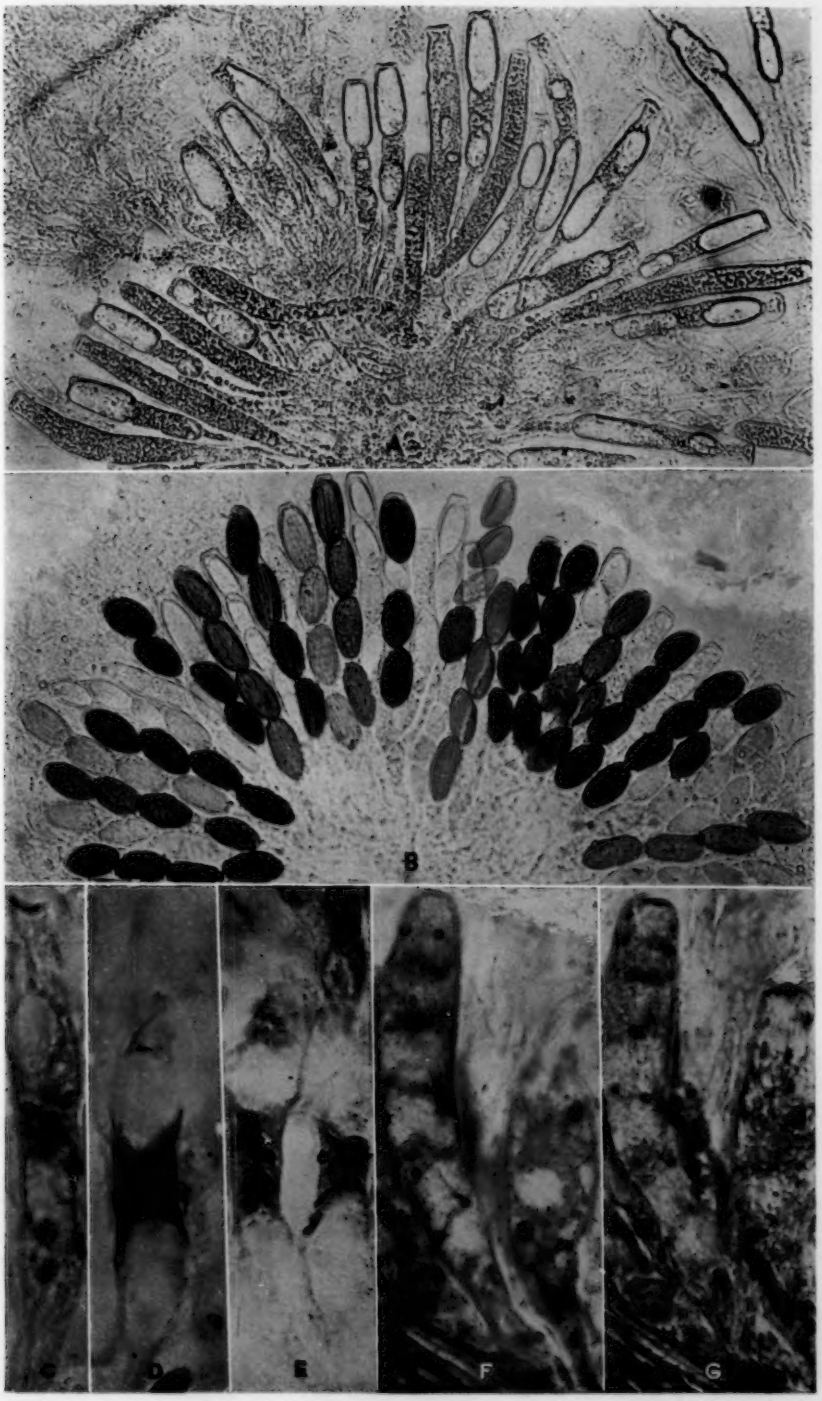
A. Young aborting asci homozygous for the recessive factor l. Compare with B below.

B. Asci heterozygous for the recessive factor l. Ascospores are delimited normally. Each spore will have originally two nuclei, one nucleus carrying l, the other nucleus of opposite sex and normal.

C–E. Photographs of stained preparations of four aborting asci homozygous for the recessive lethal l. (Compare with A above.) C, shows the eight nuclei after the third division separated into two groups by a vacuole. The vacuole in the upper end of this ascus looks like a hyaline spore. The string of cytoplasm connecting it with the tip of the ascus is not uncommon (See plate 9, C); D, E, three other asci in which the eight nuclei are crowded to the center where they are slowly degenerating. Some sections of perithecia show a dozen asci at this stage.

F, G. Two views of a section of an aborting ascus heterozygous for a lethal or deficiency due to irradiation of a parental ascospore. By focusing slightly one could see the sixteen nuclei resulting from a fourth precocious division. Their distribution and the foamy vacuolate cytoplasm are characteristic for the type of aborting asci shown in plate 9, A.


A
B
C



UNIV OF MICH

Studies in the Ericales
I. The genus Gaylussacia in North America north of Mexico

W. H. Camp[1]

In the western hemisphere, the genus *Gaylussacia* has become a depository for all the Vacciniaceae possessed of a 10-celled ovulary. This has brought together a group of plants which has but little in common in its several sections. Over forty years ago Drude[2] recognized three well defined sub-genera, namely:

(1) *Eu-Lussacia* Benth. and Hook.
(2) *Decachaena* T. and G.
(3) *Pseudo-Idaea* Dr.

In North America the species fall into the following divisions:

I. *Eu-Lussacia* Benth. and Hook. (*Lasiococcus* Small)
G. mosieri (Small) Camp.
G. orocola (Small) Camp.
G. dumosa (Andr.) T. and G.

II. *Decachaena* T. and G. (*Decamerium* Nutt.)
Sec. 1. Baccatae:
G. baccata (Wang.) K. Koch.
Sec. 2. Ursinae:
G. ursina (M. A. Curtis) T. and G.
Sec. 3. Frondosae:
G. tomentosa (Pursh) Chapm.
G. frondosa (L.) T. and G.
G. nana (A. Gray) Small.

III. *Pseudo-Idaea* Drude (*Buxella* Small)
G. brachycera (Michx.) A. Gray.

Although Small[3] has recently erected the genus *Lasiococcus* for *G. mosieri*, *G. orocola* and *G. dumosa*, the present writer is unwilling to accept the new genus until the forty or fifty species of the South American Gaylussacias are known in more detail.

For much the same reason the writer has not yet accepted Small's *Buxella*. It is to be admitted, however, that a rather cursory examination

[1] Papers from the Department of Botany, the Ohio State University, No. 332.

[2] Drude, O. Ericaceae in Pflanzenfamilien (Engler and Prantl) 4. pt. J: 49–51. 1891.

[3] Small, J. K. Manual of the Southeastern Flora. Pp. 1006–1009. 1933.

of various of the South American forms failed to locate any near relatives from which this monotypic section might have been derived.

Although it is possible that the genus Gaylussacia should be divided into three or four genera, the present writer will make no attempt to do so until more definite information of the group is at hand. Further, such a move at the present time would only be confusing to the student who would attempt to read the floristic and ecological literature of the southeastern United States where these species, under their present names, play so prominent a part.

The writer wishes to thank Mr. George Taylor of the British Museum for examining the type of *G. hirtella* (Ait.) Klotsch and also Dr. John K. Small of the New York Botanical Garden through whom the information was forwarded.

KEY TO THE SPECIES

1. Leaves evergreen, no glands on the lower surface of leaves...*G. brachycera* (Michx.) A. Gray. (Pa., E. Ky., E. Tenn., to Va., Md., and Del. Most abundant in SE W. Va.)
1. Leaves deciduous, glands present on the lower surface of the leaves.....2
   2. Glands on lower surface of leaves small, on generally curved, hair-like stalks.....3
   2. Glands on lower surface of leaves, large, short stalked and nearly sessile.....4
3. Hypanthium (receptacle, etc.) and fruit conspicuously bristly hairy with a dense cover of stalked glands.....*G. mosieri* (Small) Camp. (Fla. to La.)
   The above is incorrectly known as *G. hirtella* (Ait.) Klotsch.
   G. orocola (Small) Camp. (*Lasiococcus orocola* Small) differing from the former in being less hairy, is known from one collection made near Flat Rock, N. Car. The specimen is in the Herb. N. Y. Bot. Gard., Bronx Park, N.Y.
3. Hypanthium and fruit with inconspicuous, stalked glands—*G. dumosa* (Andr.) T. and G. (Nfd., to Fla., and La.)
   Var. *Bigeloviana* Fernald differs from the species in having the glands on the upper surface of the leaf somewhat more persistent. Mingled with the species from Nfd., southward—doubtfully distinct south of Mass., or N. J.
   4. Resin glands present and generally abundant on both surfaces of the leaf: (fruit black).....*G. baccata* (Wang.) K. Koch (Nfd., to Man., and Sask., south to Iowa, Ky., and Georgia)
      Forma *glaucocarpa* (Robinson) Mackenzie with glaucous blue fruits, sporadic, Me., to N. Car.
      Forma *leucocarpa* (Porter) Fernald with white or pinkish, translucent fruits, sporadic, throughout the range of the species.
   4. Resin glands present on lower surface only of the leaf.....5
5. Leaves with both surfaces tomentose, particularly so beneath, tomentum brown or gray, leaves leathery.....*G. tomentosa* (Pursh) Chapm. (Ga., Fla. and Ala.)
5. Leaves glabrescent or sometimes pubescent, hairs more or less silvery, leaves green or glaucous, thin or sometimes leathery.....6
   6. Leaf apex short acuminate and markedly apiculate; stamen filaments pubescent.....*G. ursina* (Curtis) T. and G (Deep forests of the Unaka Range and its outliers in the Carolinas, Tenn., and Ga.)

6. Leaf apex generally notched and revolute, occasionally acute and minutely apiculate; stamen filaments glabrous.....7
7. Mature leaves green or pale and if glaucescent, on the lower surface only.....8
7. Mature leaves with both surfaces markedly glaucescent.....9
8. Inflorescence bracts small, oblong or linear, generally deciduous..*G. frondosa* (L.) T. and G. (N. H., to Fla., and La., not present outside the Atlantic and immediate Gulf Coast drainages, erroneously reported from Ohio)
8. Inflorescence bracts large and often leaf-like, ovate to ovate-spatulate, often persistent.....*G. frondosa* var. *polycodioides* Camp. (Coastal region, N. J. and Mass.)
9. Plants low, 1–4 dm.; leaves 2–3 cm. long.....*G. nana* (A. Gray) Small. (Ga., Fla., and Ala.)
9. Plants higher, 4–10 dm.; leaves 2.5–7 cm. long.....*G. frondosa* forma *glaucophylla* Camp. (Sandy areas along the coast, Mass., to N. Car.)

Gaylussacia frondosa var. **polycodioides** var. nov.

Shrub .5–1.5 m. tall, branchlets sparsely pubescent. Mature leaves firm-membranaceous, 3–7 cm. long, 1.5–3.5 cm. wide, upper surface light green and generally glabrous except for the sparsely pubescent mid-vein; lower surface pale, glaucescent, sparsely pubescent, glandular, the margin somewhat revolute. Fruiting panicles 4–7 cm. long; *bracts semipersistent, 8–24 mm. long, 3–10 mm. wide, ovate to ovate-spatulate, subobtuse to acuminate.* Fruit frosty blue, semiglobose, 5–8 mm. in diameter, pedicels 10–18 mm. long. Flowers unknown.

Frutex 0.5–1.5 m. altus ramulis sparse pubescentibus; folia matura firme membranacea 3–7 cm. longa 1.5–3.5 cm. lata, supra pallide viridia et glabra, costa centrali sparse pubescente excepta, subtus pallida glaucescentia glandulosa, margine paullo revoluto; paniculae fructiferae 4–7 cm. longae; bracteae sub-persistentes 8–24 mm. longae 3–10 mm. latae ex ovatis ovato-spatulatae, ex subobtusis acuminatae; fructus glauco-coeruleus semiglobosus 5–8 mm. diametro, pedicellis 10–18 mm. longis; flores ignoti.

This variety, although known from only three localities, obviously belongs with *G. frondosa* but may easily be distinguished from the species by the large, leaf-like, and generally persistent bracts. The writer has chosen the name (*polycodioides*) because of the marked resemblance of the plant in the field to the deerberries (*Polycodium*). It may be easily differentiated from the associated form (*Polycodium stamineum*) by the presence of the large glands on the lower surface of the leaves.

Type locality: On the margin of a *Chamaecyparis* bog, one-half mile south of Toms River, New Jersey.

The type specimen will be deposited in the Herbarium of the New York Botanical Garden, Bronx Park, N. Y., and a co-type in the Gray Herbarium, Cambridge, Mass.

Other collections: (1) In a "Cedar swamp" near Falmouth, Barnstable Co., Mass., Sept. 24, 1915. E. Dean, A. J. Eames and L. H. MacDaniels.

Herb. Dept. of Bot., N. Y. State Coll. of Agri., Ithaca, N. Y. (2) West Tisbury, Martha's Vineyard, Mass., June 30, 1916. F. C. Seymour. Gray Herbarium, Cambridge, Mass.

Gaylussacia frondosa forma **glaucophylla** forma nov.

Shrub 4–10 dm. tall, *branchlets glaucescent.* Mature leaves firm-membranaceous to rugose, 2.5–7 cm. long, 1.3–3.5 cm. wide, *upper surface greenish-glaucescent*, often markedly so, lower surface pale glaucescent.

Frutex 4–10 dm. altus ramulis glaucescentibus; folia matura ex firme membranaceis rugosa, 2.5–7 cm. longa 1.3–3.5 cm. lata, supra viridi-glaucescentia, saepe conspicue sic, subtus pallide glaucescentia.

This form may be found in many herbaria with the species but the locality and habitat labels indicate that it is found in sandy areas close to the coast from Massachusetts to North Carolina and southward where it perhaps grades into *G. nana* (A. Gray) Small. In the mid-atlantic states, however, it may be distinguished from *G. nana* by its larger leaves and from *G. frondosa* by its highly glaucescent twigs and leaves.

SUMMARY OF NEW COMBINATIONS AND VARIETIES

Gaylussacia mosieri (Small) Camp. (*Lasiococcus mosieri* Small)
Gaylussacia orocola (Small) Camp. (*Lasiococcus orocola* Small)
Gaylussacia frondosa var. *polycodioides* Camp.
Gaylussacia frondosa forma *glaucophylla* Camp.

A study of chromosome pairing in Yucca rupicola

G. M. Watkins

(with plates 11 and 12)

INTRODUCTION

The pioneer work of van Beneden (1883) on *Ascaris megalocephala* showed for the first time that each parental nucleus contributes one basic complement of chromatin to the zygote nucleus. Overton (1893) showed that the number of chromosomes in nuclei of both male and female gametophytes of *Ceratozamia mexicana* is eight, and that double this number are found in sporophytic nuclei. In a number of other species the same relation was observed between chromosome numbers in gametophytic and sporophytic tissue. These discoveries supplemented those of Hofmeister very remarkably. Their importance was immediately recognized, and later investigators have considered from various standpoints the problem of the interrelationships of the haploid and diploid chromosome sets in the gametophytic and sporophytic phases of the life cycle. The idea of chromosome homology proposed by Montgomery (1901, 1905) and Sutton (1902) further developed the concepts in this field and gave impetus to the belief that the chromosomes which pair at the time of synapsis are really corresponding homologous members of separate gametic complements.

The work of Strasburger (1905) and the corroborative studies of many subsequent investigators have proved that the pairing of chromosomes during divisions of somatic nuclei in many plant species is a tangible reality. In *Yucca* its presence was early demonstrated by Müller (1909) in root tip nuclei in three species. In the present study I have been able to confirm the observations of Müller, and further to show that the paired relation of chromosomes is a general one which applies to all somatic cells in the adult plant. In my preparations of *Y. rupicola* pairing is completed by the time the chromosomes appear upon the equatorial plate of the first zygotic mitosis.

LITERATURE REVIEW

Montgomery's (1901) conception that each chromosome of one parental complex has its structural and physiological counterpart within the other complex was based upon his studies of division figures in diploid nuclei in which marked size differences occur among the chromosomes. He observed that in such cases there are two chromosomes of each given size, and though these two do not always appear in juxtaposition to each

other, he showed that the members of each structurally similar pair come together and fuse at the time of division of the gonotokont nuclei. Sutton (1902) found in the diploid nuclei of *Brachystola* that, although the chromosomes are not actually placed in pairs, he could arrange them in a series of pairs based on size differences. He thus observed that there are two chromosomes in each size category. A later paper by Montgomery (1905) described a partial pairing of the chromosomes of *Syrbula* in the last spermatogonial divisions.

Further progress in the development of the concept of chromosome pairing resulted from the extensive investigations of Strasburger (1905, 1907, 1909, 1910) upon various species of plants. In mitosis in root tips of *Funkia sieboldiana* and *Galtonia candicans* he observed striking size differences among the chromosomes, and that chromosomes of like size tend to pair during mitosis. He believed that the paired arrangement of homologous chromosomes is a widespread phenomenon in plants.

These researches initiated a long series of studies by other investigators on the question of chromosome pairing in somatic nuclei, and pairing was accordingly described in a wide range of plant species of many different families and orders. These data are presented in Table 1, which gives a list of plant species in which such pairing has been reported.

Clemens Müller (1909), working in Strasburger's laboratory, studied mitosis in root tips of three species of Yucca (*Y. aloifolia*, *Y. draconis*, and *Y. guatemalensis*). According to his account the three species are cytologically identical and are characterized by the presence in diploid nuclei of ten long, club-shaped chromosomes and about forty-four to forty-six which are very small and almost spherical. The large chromosomes are almost always arranged in five pairs, and the small ones also tend to pair. Müller followed the progress of the chromosomes in root tip cells from their first emergence out of the resting nuclear reticulum through all the phases of mitosis until their final disappearance in the late telophases. In every phase where the chromosomes are clearly visible they are arranged in pairs. Müller's contentions are well supported by excellent photographs of his preparations as well as numerous drawings.

Shortly afterward Bonnet (1911) contradicted Müller's views, stating that in somatic divisions of *Yucca* the chromosomes are not paired, and that any such appearance is due entirely to a fortuitous side-by-side arrangement. In my opinion Bonnet's criticism, which was based on his observations of mitosis in *Y. gloriosa*, carries no great weight, since his material was so poorly fixed that the chromosomes appear as little more than a fused chromatin network lying in the equatorial plane.

Müller further substantiated his views in a second paper (1912) dealing

TABLE 1

SPECIES	FAMILY	AUTHORITY
Morus indica	Moraceae	Tahara, 1910
Cannabis sativa	Moraceae	Strasburger, 1910
Spinacia oleracea	Chenopodiaceae	Stomps, 1910
Lychnis dioica	Caryophyllaceae	Sykes, 1908
Pisum sativum	Leguminosae	Strasburger, 1907
Mercurialis annua	Euphorbiaceae	Strasburger, 1910
Ricinus zanzibariensis	Euphorbiaceae	Nemec, 1910
Mouriria anomala	Melastomataceae	Ruys, 1924, 1925
Nicandra physaloides (paired prochromosomes)	Solanaceae	Janaki-Ammal, 1932
Plantago lanceolata	Plantaginaceae	Nemec, 1910
Bryonia dioica	Cucurbitaceae	Sykes, 1908
Dahlia spp.	Compositae	Ishikawa, 1911 Lawrence, 1929
Naias marina	Naiadaceae	Müller, 1912
Hydrocharis morsus-ranae	Hydrocharitaceae	Sykes, 1908
Bulbine annua	Liliaceae	Müller, 1912
Funkia sieboldiana	Liliaceae	Strasburger, 1905 Sykes, 1908 Müller, 1912
Funkia ovata	Liliaceae	Sykes, 1908
Aloë hanburyana	Liliaceae	Müller, 1912
Albuca fastigiata	Liliaceae	Müller, 1912
Galtonia candicans	Liliaceae	Strasburger, 1905 Sykes, 1908 Müller, 1912
Eucomis bicolor	Liliaceae	Müller, 1912
Muscari botryoides	Liliaceae	Müller, 1912
Scilla bifolia	Liliaceae	Müller, 1912
Chionodoxa lucillae	Liliaceae	Müller, 1912
Hyacinthus orientalis	Liliaceae	Müller, 1912
Yucca aloifolia	Liliaceae	Müller, 1909, 1912
Yucca draconis	Liliaceae	Müller, 1909, 1912
Yucca guatemalensis	Liliaceae	Müller, 1909, 1912
Beschorneria superba	Amaryllidaceae	Müller, 1912
Nerine rosea	Amaryllidaceae	Müller, 1912
Oryza sativa	Gramineae	Kuwada, 1910
Holcus halepensis	Gramineae	Huskins and Smith, 1932
Listera ovata	Orchidaceae	Müller, 1912

Table 1—Plants in which pairing of chromosomes in divisions of somatic nuclei has been reported.

with chromosome arrangement in fourteen genera of the Liliaceae, Amaryllidaceae, Orchidaceae, and Naiadaceae (see table 1). In all of the plants studied he demonstrated a definite tendency for the chromosomes to pair in diploid mitoses.

Additions to the data relating to chromosome pairing were made

by Tahara (1910) on *Morus indica*, Kuwada (1910) on *Oryza sativa*, and Ishikawa (1911) for various species of *Dahlia*. In these cases the chromosomes were said to be paired in somatic mitoses and also in the homoeotypic divisions of microsporogenesis. Ishikawa offered the explanation that these forms are really tetraploid in the sporophytic generation and that the reduced number of chromosomes found in the homoeotypic divisions and in the gametophytic nuclei nonetheless contains two basic complements. Hence, any tendency toward pairing of homologues can well be expressed even in homoeotypic divisions. This peculair type of chromosome arrangement, to which Darlington (1928) has applied the term "secondary pairing," has also been figured and described for *Mercurialis annua* by Yampolsky (1925).

The work of Montgomery (1901) and Sutton (1902) provided the stimulus for a series of investigations on the occurrence of chromosome pairing in non-meiotic divisions in animal cells. Miss Stevens (1908) described chromosome pairing for all spermatogonial divisions of *Culex*. Her observations were substantiated by Metz (1914, 1916) in a series of studies on chromosomes in approximately eighty species of the Diptera. The association in distinct pairs of the homologues in various somatic and spermatogonial nuclei of the flies, as described by Metz, is perhaps the most perfect and diagrammatic so far reported for any organism. As a rule the Diptera seem to have comparatively low chromosome numbers and also to combine several size categories for each species, and the phenomena described by him appear to be general for the entire order.

Metz and Nonidez (1921, 1923) described spermatogonial and meiotic divisions in *Asilus sericeus* and *A. notatus*. In the last spermatogonial division of *Asilus*, according to their description and figures, the pairing of the homologous chromosomes in the telophases is so intimate that it takes the place of synapsis. No leptotene stage is present in the meiotic prophases and the chromosomes appear immediately as greatly condensed bivalents. A similar condition was observed in *Dasyllis grossa* (Metz, 1922b). Whiting (1917) described conditions for *Culex* which agree essentially with those figured by Metz for the order as a whole.

Robertson (1930), studying chromosomes of both parthenogenetically produced and biparental individuals of *Apotettix eurycephalus* and *Paratettix texanus*, found that in dividing nuclei of the former class, the chromosomes are closely paired, but in biparental individuals the chromosomes of the gametic sets appear on opposite sides of the spindle. He suggests that the partheno-produced individuals may have arisen from the type of egg which has a single pronucleus containing seven double or diad-like chromosomes, and that the biparental form comes from an egg with two

pronuclei, each of which contains seven single or monad-like chromomoses. This would account for the appearance of the same chromosome numbers in both types, and it is offered by Robertson as an explanation for the interesting variation from chromosome pairing to gonomery within the same species.

As a corollary to his idea that homologous chromosomes tend to pair in diploid somatic nuclei Strasburger (1907) suggested that in polyploid nuclei the chromosomes might be found aggregated in still larger groups than pairs. He investigated dividing nuclei in endosperm tissue, which is triploid, and analyzed syndiploid nuclei of chloralized pea root tips to to determine whether groups of four are present. He never observed these, however, and concluded that the affinity which homologous chromosomes have for each other is satisfied when two homologues pair, and that no tendency to form larger groups is present. Strasburger's figures of equatorial plates of syndiploid pea root tip nuclei show a pronounced pairing, but no evidence of grouping in fours.

This aspect of the problem has received little further study and the data available do not warrant generalizations. Ruys (1924, 1925), on the other hand, has figured two division stages of endosperm nuclei in *Mouriria anomala* which showed the chromosomes arranged in groups of three. The haploid number in this species is twelve, and Ruys observed in the metaphase figure twelve groups of three chromosomes each. In diploid equatorial plate figures in nucellar tissue he observed twelve pairs. Metz (1922a) reported divisions of certain tetraploid somatic nuclei in *Sarcophaga*, a dipteran, in which the haploid number is six, and division figures are given showing six groups of four chromosomes each. Miss Holt (1917) described various complex nuclei in *Culex;* these ranged from diploid to polyploids with twenty-four basic sets of three chromosomes per set. In the less complicated polyploid nuclei her figures show the homologues grouped together, but in the higher polyploids the association is not realized.

The data accumulated since 1905 are sufficient to indicate strongly that the phenomenon of chromosome pairing in nuclei of somatic tissue is clearly defined and tangible for a large number of plant species as well as for at least one order of animals. Almost all data, however, relate to cells in isolated embryonic zones in the more or less mature organism. These cells are many mitotic generations removed from the point of initiation of the diploid state, and hence they throw no light on the time and manner of origin of the paired condition. In his various papers Metz has shown that the chromosomes are paired in the cells of very young dipteran larvae, but even these stages are far later in development than the point where

the relationship might possibly begin. Huettner (1923), in a study of the origin of germ cells in *Drosophila melanogaster*, found that after fertilization the nuclei during the fifth, sixth, and seventh cleavage stages are located at the end of the egg in a zone of granular polar cytoplasm. During these nuclear divisions Huettner observed that the chromosomes are paired. In a later paper (1924) he showed that at syngamy in the same organism the male and female pronuclei approach each other in the resting condition and without fusing proceed to the formation of gonomeric spindles side by side. Homologous chromosomes do not mingle, and pairing of the homologues occurs for the first time in the second cleavage.

According to the account given by Hutchinson (1915) for fertilization and division of the zygote nucleus in *Abies balsamea*, the egg and sperm nuclei fuse in the resting condition. Following this two groups of chromosomes arise in the micropylar end of the fusion nucleus, and as the first division spindle is set up the two groups become intermingled at the equatorial plate with the chromosomes arranged in pairs. Each pair undergoes transverse segmentation and the daughter segments, still paired, go to opposite poles and the daughter nuclei are reconstructed. A similar process was described by Chamberlain (1916) for fertilization in the cycad, *Stangeria paradoxa*, and by Miss Weniger (1918) for *Lilium philadelphicum* and *L. longiflorum*. These three accounts have been criticized by Sax (1918), because the reported transverse segmentation of the chromosomes is not in line with the more widely accepted conceptions of the manner of distribution of the daughter chromatin elements in mitosis.

The published data relating to the pairing of chromosomes in somatic cells of *Yucca* are limited to the papers of Clemens Müller (1909, 1912) and Bonnet (1911) previously mentioned. Other cytological details, as presented by various authors for a number of species of the Yucceae, are listed in table 2.

MATERIALS AND METHODS

I have studied especially *Yucca rupicola* Scheele. Cytological material was collected in the field at Austin, Texas, during flowering time through three successive summers. Preliminary fixations were made in a variety of solutions, and from a study of these the Allen and Wilson modification of Bouin's solution was selected for the preservation of large numbers of young flowering buds, slices of developing ovaries after pollination, and young ovules in various stages of development. Seeds were germinated in sand and between sheets of moist filter paper, and when the seedling roots were from one to three centimeters long the tips were removed, rinsed

briefly in the case of those grown in sand, and fixed in the modified Bouin's solution. All material was imbedded in paraffin, sectioned at varying thicknesses from 7 to 15 microns, and stained with the Flemming triple stain or by the Heidenhain iron alum-haematoxylin method.

TABLE 2

SPECIES	CHROMOSOME NUMBERS N	2N	AUTHORITY
Hesperaloë parviflora	30	—	McKelvey and Sax, 1933
Hesperoyucca whipplei	30	—	McKelvey and Sax, 1933
Samuela faxoniana	30	—	McKelvey and Sax, 1933
Yucca aloifolia	—	54–56	Müller, 1909, 1912
Y. angustissima	30	—	McKelvey and Sax, 1933
Y. constricta	30	—	McKelvey and Sax, 1933
Y. draconis	—	54–56	Müller, 1909, 1912
Y. elata	30	—	McKelvey and Sax, 1933
Y. filamentosa	—	—	Koernicke, 1901
	—	—	Taylor, 1925
	30	—	Morinaga, *et al.*, 1929
	30	—	McKelvey and Sax, 1933
Y. flaccida	30	—	O'Mara, 1931
	30	—	McKelvey and Sax, 1933
Y. glauca	6	12	Folsom, 1916
Y. gloriosa	—	—	Bonnet, 1911
Y. guatemalensis	—	54–56	Müller, 1909, 1912
Y. macrocarpa	30	—	McKelvey and Sax, 1933
Y. recurva	ca. 25	54–56	Woycicki, 1911, 1925
Y. rupicola	30	—	McKelvey and Sax, 1933

Table 2—Chromosome studies in the Yucceae.

OBSERVATIONS

Mitosis in root tips

Resting nuclei in the root tips usually show a single nucleolus surrounded by a very finely drawn chromatic reticulum. In many cases a clear space lies between the nucleolus and the chromatin, a characteristic which is shown in the photographs by Müller (1909). As the prophases begin the chromatin becomes aggregated into densely staining discrete chromosomes which show no evidence of forming a continuous spireme. At this stage it becomes apparent that there are two sizes of chromosomes, one group of long, rod-like chromosomes and another of short, almost spherical ones. Chromosomes of both sizes are completely intermingled and show no specific plan of orientation. At this stage definite pairs of chromosomes are seldom visible.

As the nuclear membrane breaks down and the nucleolus disappears the central spindle is formed with the chromosomes distributed across the plane of the equatorial plate. The distribution of these chromosomes corresponds closely with previously published accounts (e.g., Strasburger, 1905; Miyake, 1905; and Müller, 1909, 1912) of equatorial plate figures in species which have chromosomes of markedly different sizes. That is, the small ones occupy the central area and the large chromosomes radiate out peripherally from the margin of the spindle. In figures 1, 2, and 3 are shown polar views of such equatorial plates. Careful counts of a large number of plates indicate that there are ten large chromosomes and fifty small ones. The typical radial placement of the long chromosomes may be varied occasionally by the vertical orientation of one or more pairs. The reason for these exceptions is not apparent, but it is to be noted that the spindle fiber attachments of all the long members are terminal, and that the distal portions of the chromosomes are free to become oriented by other agencies than the spindle fibers themselves. In other words, each long member of the chromosome complex appears to pivot about a locus established by the spindle attachment.

In the majority of equatorial plate stages examined a marked tendency toward pairing of the chromosomes is evident. Figures 1, 2, and 3 are typical of the condition described. With the view of emphasizing more objectively the long chromosomes which appear to constitute a pair they have been indicated alphabetically in these figures. The pairing is not sufficiently intimate and close to suggest synaptic pairing or chromosome conjugation in meiosis, but it is, nevertheless, easy in most cases to recognize for each long chromosome a definite partner or mate, not only because of similarity in size and shape, but also through its close proximity and similarity in direction of orientation. Pairing is also evident in a large number of small chromosomes, but their great number and close crowding render the relationship less obvious than in the former group. Contrary to the findings of Müller for *Y. aloifolia*, *Y. draconis*, and *Y. guatemalensis*, I have been able to demonstrate pairing clearly in only the equatorial plate stages of root tip mitoses. His figures show pairing through mitosis from the prophases to the telophase condition. In figure 4 is shown an anaphase typical of root tip mitoses in *Y. rupicola*. Doubtless the tendency to pair is here just as strong as in the equatorial plate, but because of the crowding of the chromosomes as they approach the poles, it is more difficult to recognize. At the stage shown in figure 4 the small chromosomes may often be so crowded as to make identification of the individuals impossible. Nevertheless the long chromosomes in this anaphase figure are arranged in fairly definite pairs.

Mitosis in ovarian tissue

In the walls of young ovaries nuclear division is fairly frequent and various stages of mitosis can readily be observed. A careful study of these stages shows them to be similar in every detail to the corresponding processes in root tips. In equatorial plate stages the long chromosomes are paired and are radially placed, with the small ones in the central part of the figure. Pairing is conspicuous, especially among the long chromosomes, in most nuclei at this stage, as is shown in figures 5 and 6. Prophases and telophases failed to show consistent evidence of chromosome pairing, possibly due again to the difficulty of analyzing the relationships existent within them.

Mitosis in young embryos

Despite the very great difficulty involved in the location of clear equatorial plate figures in young embryos of *Yucca*, a sufficient number of mitoses have been found in the early embryonic stages to give a clear picture of the arrangement of the chromosomes during this part of the sporophytic development. In figure 7 is shown a polar view of an equatorial plate which is typical of several such stages observed in an embryo containing probably between five hundred and a thousand cells. Many of the smaller chromosomes are omitted from this drawing, due to their overlapping and the consequent difficulty in determining their exact number and positions. The large chromosomes lie in their characteristic positions and are definitely paired. A younger embryo is shown in figure 12. It contains a filament of five cells, the central one of which contains an equatorial plate stage. This figure, although seen in profile view, is so clear that it is easily possible to follow the exact positions of the long chromosomes, and to reconstruct from it an orthographic projection showing the appearance of the plate from the apical pole, as illustrated in figure 11. Figure 13 is an enlarged camera lucida drawing of the original mitotic figure. Here is a clear cut case of pairing of the long chromosomes. Pairs "*a*", "*b*", "*c*", and "*d*" show the typical radial arrangement previously described, while pair "*e*" lies in the vertical direction at right angles to the equatorial plate.

The evidence as to the pairing of the chromosomes during the early prophases of mitosis in these filamentous embryos is so far indecisive, because of the limited number of such stages found. Several prophases have been studied in which the chromosomes appeared thoroughly intermingled and with no obvious paired arrangement. A few figures were found, however, in which several chromatic units were seen lying side by

side, as is shown in figure 10. The paired arrangement of the chromatin rods in this figure indicates that pairing is achieved in the late prophases, but it is not certain at what stage in mitosis it becomes conspicuous.

In figure 8 is shown an oblique view of an equatorial plate from an embryo in the three- or four-celled stage. All of the long chromosomes can be seen clearly and they are definitely assorted in pairs. The embryo shown in figure 9 consists of three cells, the apical of which is in the telophase of mitosis. Reorganization of the daughter nuclei has proceeded to a considerable extent and the chromosomes have become almost completely transformed into a characteristic interkinetic reticulum.

A series of stages showing some of the steps in mitosis in the first division of the fertilized egg or one-celled embryo are illustrated in figures 14, 15, 16, and 17. Unfortunately the embryo shown in figure 14 was cut through by the knife, but, since the chromosomes appear to be undisturbed, it was deemed worth while to draw separately each part of the embryo as it appeared in the serial sections. The prophase chromosomes appear as discrete, thickened bodies in this stage. Although there seems to be sufficient space in the nucleus to allow the chromosomes to assume almost any grouping, they are irregularly distributed and no pairing is evident.

Figure 15 shows an equatorial plate of the first embryonic mitosis. This figure is unusual in that it shows three of the five pairs of chromosomes oriented vertically, leaving only two pairs lying in what has been postulated as the typical radial orientation. Of these latter two pairs one is shown heavily shaded (pair "*a*") and extends obliquely upward from the spindle. The other radially disposed pair cannot be indicated in this drawing, due to the fact that it extends straight downward beneath the mass of small chromosomes. The pair can be accurately determined in the preparation by focusing slowly downward. The paired arrangement of the large chromosomes in this figure is clear and well defined.

A slightly later stage is shown in figure 16. This is a very early anaphase stage and shows the chromosomes just after separation of the daughter halves. The long chromosomes are still pointed outward and poleward much as they are on the equatorial plate, and the distal portions have not yet manifested the lagging equatorial orientation which characterizes them in later anaphases. Pairing of the chromosomes is not conspicuous in this figure. In a somewhat more advanced anaphase, such as the one shown in figure 17, the long chromosomes are trailing behind the general group as the poleward movement proceeds. In this figure there is some evidence of pairing.

The shape of the achromatic figure becomes much changed as the

anaphase occurs. At the equatorial plate stage (fig. 15) the spindle is fairly broad and the fibers become indistinct before converging at the poles. The same applies to the early anaphase (fig. 16). But during later anaphases the spindle becomes extended longitudinally and the fibers converge into sharp, distinct points at the poles (fig. 17).

Nuclear division in the endosperm

In addition to the observations described above a study was also made of the free nuclear divisions which occur during the early development of the endosperm, with the purpose of determining whether the same affinity of homologous chromosomes found in diploid nuclei is manifested also in the endosperm, which presumably is triploid. In view of the fact that the triple fusion of a sperm cell with the two polar nuclei has so far not been observed in *Yucca*, it is not certain that the endosperm nuclei studied have arisen in the normal manner. Chromosome counts in the endosperm have yet failed to show the theoretical triploid number, i.e., ninety, but a number of figures have been studied in which about seventy-five chromosomes were recognizable. Perfect nuclear fixation is an absolute requirement for the accurate study of nuclei with such high chromosome numbers, and this is obtained only with the greatest difficulty in the endosperm, which is surrounded by many layers of cells.

Figures 18 and 19 show typical late prophases from the endosperm. No definite arrangement of the chromosomes in groups of three is visible in these nuclei, and the same irregular intermingling of chromosomes is evident in the equatorial plate stages of figures 20 and 21. There is here no visible tendency of the chromosomes to be arranged in groups of three. These observations are perhaps in accord with the assumption of Strasburger that when two chromosomes pair their mutual affinities are satisfied and there is no further tendency toward the formation of larger aggregations. In endosperm nuclei there is little or no evidence at hand that the homologues are arranged in any specific manner with reference to each other. In general, as shown in figure 21, the long chromosomes tend to radiate out from the margin of the chromosome group with the small chromosomes occupying the center.

In a recent study of chromosomes in the hemipter, *Archimerus alternatus*, Wilson (1932) has described the preservation of a so-called stable metaphase pattern in haploid, diploid, and tetraploid nuclear divisions. Regardless of the number of basic chromosome complexes present the autosomes tend to form a ring, with the small m-chromosomes in the center and the x-chromosomes on the outside. This is considered evidence for the view that each chromosome, together with its allotment of achromatic

material, is an autonomous unit within the nucleus, which takes a specified position upon the spindle regardless of additional complications such as the reduplication of chromosome complexes. The view that there is a permanently fixed relation between the chromosomes and the achromatic material was early presented by Rabl (1885) for animal nuclei and by Harper (1896, 1905) in his work on nuclear division in *Erysiphe* and *Phyllactinia*. Bleier (1930) has recently subscribed to this view in connection with his discovery of a type of gonomery in meitoic divisions in pollen mother cells of *Triticum* x *Secale* and *Aegilops* x *Triticum* hybrids. Bleier's term for this feature is "Krypto-gonomerie," and he employs the term "paragenoplast" for the allotment of achromatic material which goes with each chromosome.

Yucca also preserves the same equatorial plate pattern in haploid, diploid, and possibly triploid mitoses; the large chromosomes are radially placed at the periphery of the figure, while the small ones occupy the central position. Haploid figures conforming to this scheme have been presented by Morinaga *et al.* (1929), O'Mara (1931), and McKelvey and Sax (1933), diploid figures by Müller (1909), and both diploid and triploid are here presented in figures 1, 2, 3, 5, 6, 7, 8, 11, 20, and 21.

DISCUSSION

In the majority of papers dealing with the subject of chromosome pairing in diploid nuclei the basic assumption is that the members of each pair are derived from the paternal and maternal parents respectively. Montgomery's contribution was primarily a suggestion of the homology of chromosomes which fitted admirably with the facts at hand, and the strength of that suggestion is attested by the fact that it is still applicable to the data accumulated in the three decades since its proposal. However, the organisms which he studied showed chromosome pairing only at synapsis or during the last spermatogonial division, thus leaving a gap of many cell generations during which visible evidence of the homology had not been traced. Strasburger and his students narrowed the gap considerably by their studies of species which show chromosome pairing in diploid somatic nuclei, root tips chiefly, but these data do not include the processes of syngamy or the early stages in the development of the sporophyte. Müller's excellent work on *Yucca* refers to karyokinesis in root tips, and his conclusive evidence of chromosome pairing in such nuclei was not only strong support for the theory of chromosome individuality, but also substantial correlative evidence that the members of each pair are homologous.

My data are further and more conclusive visible evidence of this

homology, since it has been shown that in *Yucca* the chromosomes occur in pairs in diploid somatic nuclei in various parts of the plant, and in young developmental as well as in more mature stages. The chromosomes, according to my observations so far, first appear in pairs upon the equatorial plate of the first zygotic division spindle and continue thus throughout the development of the sporophyte. Further data as to chromosome homology may come from a careful serial study of gametic karyogamy to determine when and how the assortative matings of the chromosomes come about.

The work of Huettner (1924) offers to the solution of this problem perhaps the most specific data to be found among the investigations in animal cytology, since he has demonstrated that in *Drosophila melanogaster* the beginning of the paired relationship takes place sometime in the interphase between the telophases of the first gonomeric cleavage division and the metaphases of the second divisions, where the chromosomes are first seen in pairs.

In comparing diploid equatorial plates of *Yucca* with those of other organisms in which pairing of chromosomes in somatic divisions has been described, striking differences in the intimacy of the association are at once noted. These may perhaps be due to variations in affinity between the members of the different pairs. In general it may be said that the intimacy of the pairing is more evident as described in the Diptera than in most of the plants studied. Heitz and Bauer (1933) and Painter (1934) have recently reported synapsis in somatic nuclei in certain species in this order. Heitz and Bauer found typical synapsis figures in cells of ovarian follicles and the malpighian vessels in *Bibio hortulanus*, and Painter has described a similar chromosome conjugation in the salivary glands of larvae of *Drosophila melanogaster*. Additional evidence of the close relationship between homologues in the Diptera is seen in the tendency of the chromosomes to aggregate in larger groups than pairs in the higher polyploids. As previously mentioned the presence of larger aggregates is as yet not well authenticated for plants.

As Miss Fraser (1912) has pointed out, the various groups of organisms may differ widely with respect to the behavior of gametic chromatin after fertilization. On this basis it is possible to arrange some of the known cases of fertilization in a series based on the rapidity with which parental chromosomes become associated together and also upon the closeness of this association. First in this series, so far as known, is *Yucca*, since pairing of homologous chromosomes occurs by the time they appear upon the equatorial plate of the first zygotic division. Close to *Yucca* would come *Drosophila melanogaster*, for Huettner's (1924) account has shown that

the paired relationship begins with the second embryonic mitoses. Next in the series would come the several plant species, such as *Pisum sativum*, *Mouriria anomala*, and *Galtonia candicans*, which show a pairing of chromosomes in somatic divisions, but in which the pairing has so far been demonstrated only in the adult plant. In none of these forms has the pairing in somatic nuclei been adequately shown to preclude the existence of synaptic pairing. The next group in order is the large class into which nearly all plant and animal species fall, as judged by the literature. In these there is complete karyogamy at the time of fertilization, but no obvious pairing of parental chromatin units until near the end of the diploid phase, that is, during meiosis. A further departure is found in *Pinus* (Ferguson, 1904), which behaves in the same manner as the last group except for the manifestation of gonomery in the first division of the sporophyte. The cases of *Cyclops* (Rückert, 1895; Häcker, 1895) and *Cryptobranchus* (Smith, 1919) are still farther removed, because in them the condition of gonomery persists for a varying number of cell generations after fertilization.

As the other extreme in this series we find the condition existing in the rusts and some other groups of fungi. In the rusts fertilization seems to consist of a type of plasmogamy which is not accompanied by karyogamy. As this diploid phase progresses the two gametic nuclei are found closely associated as a dikaryon. They undergo conjugate nuclear divisions for a period until the teleutospore stage is reached. Then nuclear fusion occurs, only to be followed immediately by meiosis and a return to the haploid condition. Far from the state found in *Yucca* these rusts seem to show entire independence of the parental chromosomes throughout almost the entire diploid phase of the life cycle.

Winge (1917) has recognized the problem of the varying behavior of gametic chromatin following syngamy in different organisms, and has attempted to classify and label from this standpoint three types of gametic activity. His term "philozygoty" refers to the union of gametes which are in complete harmony with each other, such as gametes from the same species. The harmony may be such as to result in pairing of chromosomes, although Winge states that this is often first visible in the sporogenous cells. By definition the application of the word "philozygoty" is too vague to be useful for distinguishing members of the series which has been postulated above, because all the forms listed, even with their widely varying degrees of gametic harmony, would be included in this category. Winge's other terms, "pathozygoty" and "misozygoty," refer to gametic relationships between hybrid parents in which syngamy is accomplished under very abnormal circumstances, or not at all.

This study has been carried on under the direction of Professor R. A. Harper and Professor J. S. Karling, whose continued encouragement and advice I gratefully acknowledge.

SUMMARY

1. Müller's report of pairing of chromosomes in root tip mitoses in three species of *Yucca* is here confirmed for *Yucca rupicola* Scheele.

2. Ten large and fifty small chromosomes are present in each diploid nucleus.

3. Chromosome pairing is likewise shown to be present in other embryonic zones, such as developing ovaries.

4. In the first division of the zygote nucleus and in later mitoses in developing embryos the chromosomes are arranged in pairs.

5. No definite arrangement of homologous chromosomes was observed in the endosperm nuclei.

6. If organisms are classified on the basis of the promptness with which their gametic complements come together after the beginning of the diploid state, *Yucca* is so far one extreme in such a series.

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Explanation of plates 11 and 12

In studying the preparations a Bausch and Lomb microscope was used. Figures were drawn with a camera lucida, using the 100× oil immersion objective and the 12.5× and 7.5× oculars. The magnification of all figures is 2300, except figures 9 and 12, which are magnified 1250 times.

Figs. 1, 2, and 3. Polar views of equatorial plate stages from root tip.

Fig. 4. Anaphase from a root tip cell.

Figs. 5 and 6. Polar views of equatorial plate stages from developing ovary.

Fig. 7. Polar view of an equatorial plate from an embryo of 500 to 1000 cells.

Fig. 8. Oblique view of an equatorial plate from an embryo of three or four cells.

Fig. 9. A three celled embryo, showing a telophase stage of mitosis in the terminal cell.

Fig. 10. A prophase stage from a cell of a young filamentous embryo.

Fig. 11. Reconstructed polar view of the equatorial plate stage shown in the median cell of the embryo shown in fig. 12.

Fig. 12. A five-celled embryo.

Fig. 13. An enlarged drawing of the median cell of the embryo in the preceding figure.

Fig. 14 (a and b). An embryo of one cell in a late prophase of the first embryonic mitosis. The cell was cut in the sectioning and hence appears in adjacent sections on the slide.

Fig. 15. The equatorial plate stage of the first embryonic mitosis.

Fig. 16. Early anaphase of the first embryonic mitosis.

Fig. 17. Later anaphase of the first embryonic mitosis.

Fig. 18. Late prophase of free nuclear division in endosperm.

Fig. 19. Later prophase of endosperm mitosis after the nuclear membrane has disappeared.

Figs. 20 and 21. Equatorial plate figures in the endosperm.

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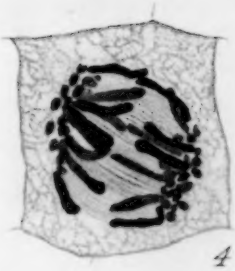

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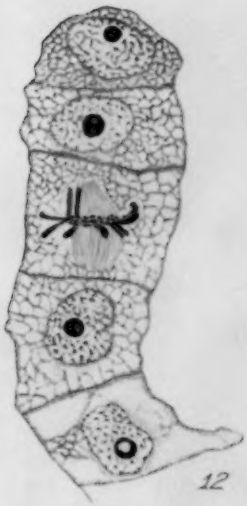

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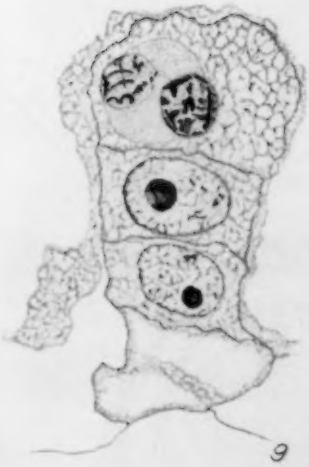

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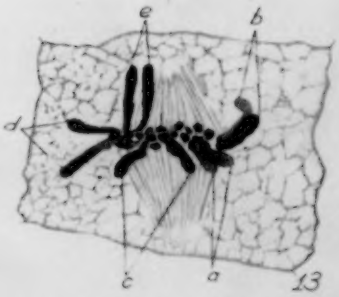

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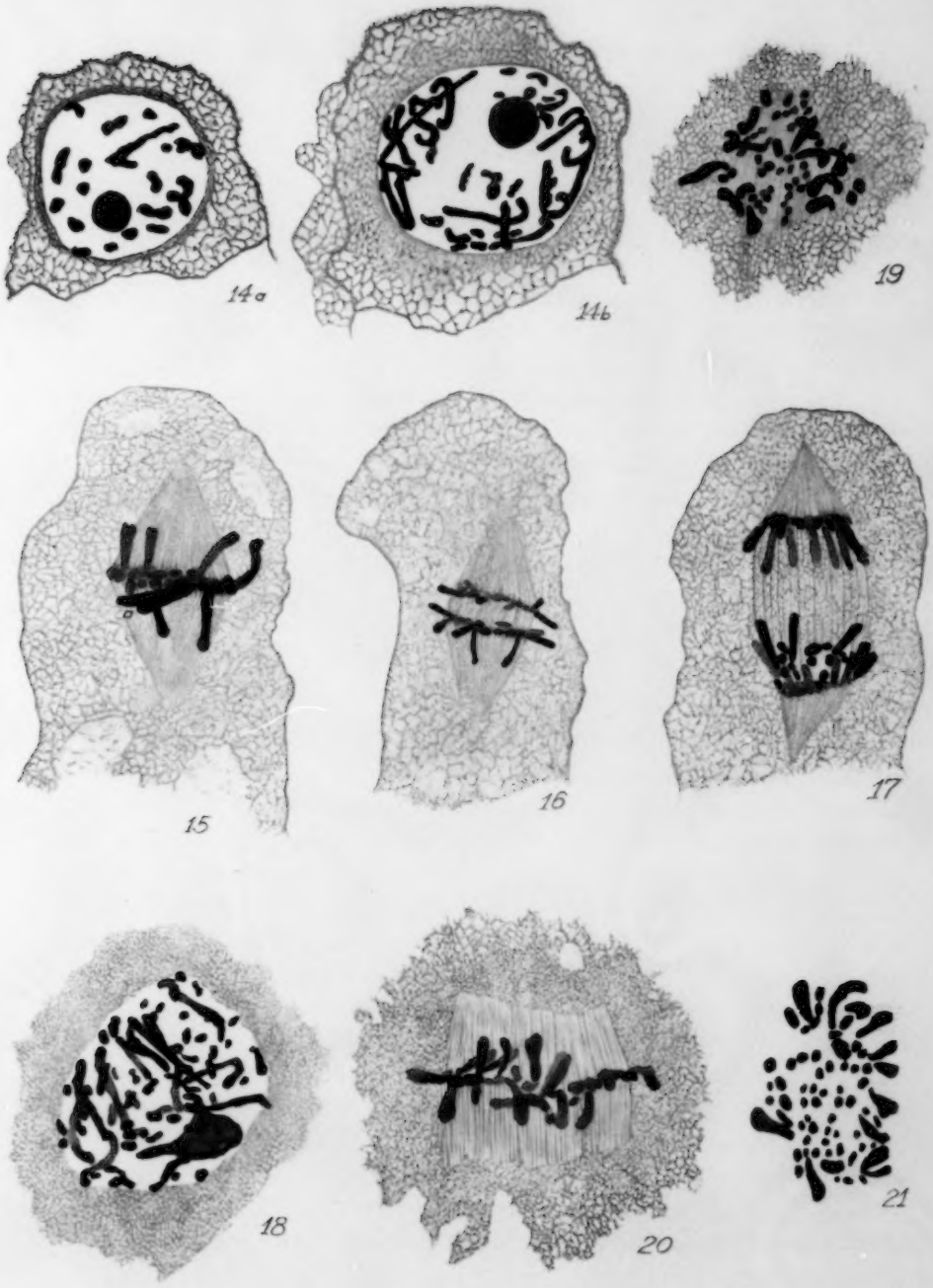
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Effects of ultra-violet radiation and temperature on Fusarium
II. Stimulation

ELIZABETH C. SMITH

(WITH FOUR FIGURES)

INTRODUCTION

The phenomenon of stimulation has been in dispute for some time. It has been considered by many as an irregular sort of process whose occurrence is unpredictable. Because of this irregularity the subject has aroused considerable interest, and for the same reason data concerning it are very meager in the literature. The data which will be presented in this paper indicate that at least in certain cases stimulation is predictable and its magnitude can be controlled by controlling certain environmental factors.

The lethal action of ultra-violet radiation and temperature on *Fusarium Eumartii* Carp. has already been discussed (Smith, 1935). The same species has been used in a study of the stimulation of vegetative growth and of spore production.

STIMULATION OF VEGETATIVE GROWTH

Methods

A suspension of spores was made in sterile distilled water. The spore load (the number of spores in 1 cc. of water) was determined with a Levy counting chamber. Enough of the suspension was added to sterile distilled water to give a spore load of not more than 40. One cubic centimeter was then plated out on a soft gelatin medium. The medium was prepared as follows: 200 grams of fresh potato slices were placed in 500 cc. of distilled water and the mixture was autoclaved at 15 pounds pressure for 20 minutes. One hundred grams of gelatin were dissolved in 500 cc. of warm distilled water. The two mixtures were combined, 30 grams of dextrose were added and the solution was diluted with distilled water to 1 liter. The pH was adjusted to 6.6 by the colorimetric method. The medium was sterilized in an Arnold steam sterilizer for 30 minutes on two successive days. About 72 hours after plating out the spores the colonies were large enough to transplant to agar. A scalpel dipped in alcohol and flamed was used to make the transfer. Single colonies were picked up with the tip of the scalpel and transferred in a sterile chamber to agar plates. Because of the softness of the gelatin medium only a small amount of gelatin adhered to the colonies. Three colonies were placed in each Petri dish. The agar used was a potato-dextrose agar. The preparation of this has already been described (Smith, 1935). For one experiment a so-called "poor" agar was

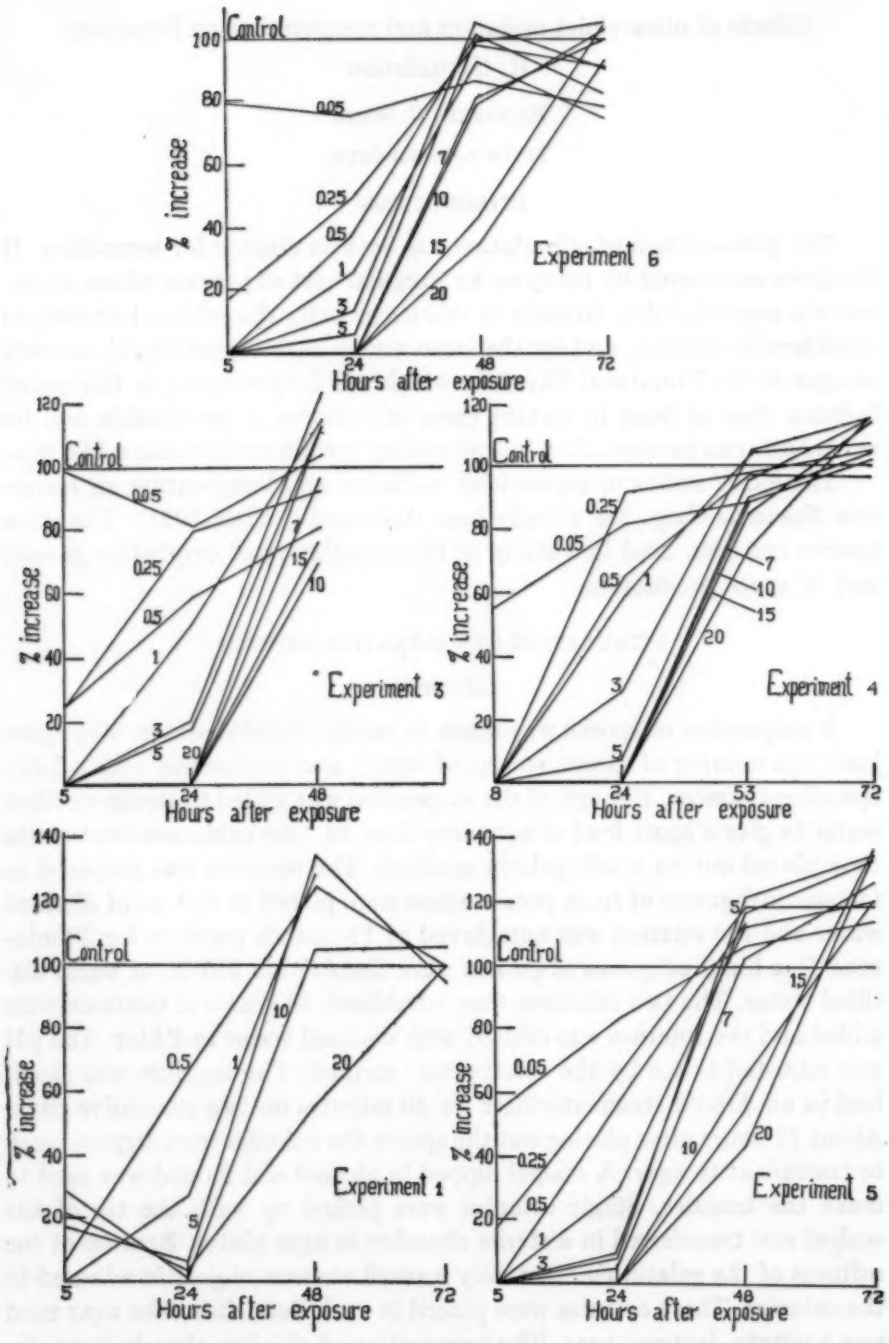

Fig. 1. Stimulation of vegetative growth as shown by the comparative growth rates of irradiated and control cultures during various intervals of time following irradiation. The period of irradiation in minutes is indicated on each curve.

TABLE 1

The mean increase in diameter (in mm. and as % of control) of irradiated and control cultures during various intervals of time following irradiation.

EXP. NO.	MINUTES EXPOSURE	0–5 HRS		5–24 HRS.		24–48 HRS.		48–72 HRS.	
		AV	%	AV.	%	AV.	%	AV.	%
1	Control	2.12	100.0	6.00	100.0	7.37	100.0	8.75	100.0
(12)*	0.5	0.12	5.6	4.12	68.6	9.25	125.5	8.75	100.0
	1	0.00	0.0	1.75	27.2	9.50	128.8		
	5	0.37	17.4	0.37	6.1	10.00	135.6	8.33	95.2
	10	0.62	29.2	0.12	2.0	7.75	105.3	9.25	105.7
	20	0.45	21.2	0.25	4.1	4.87	66.0	8.75	100.0
3	Control	1.34	100.0	5.52	100.0	7.17	100.0		
(33)	0.05	1.33	99.2	4.51	81.7	7.45	103.9		
	0.25	0.36	26.8	4.45	80.6	6.65	92.7		
	0.5	0.35	26.1	3.25	58.8	5.76	80.3		
	1	0.00	0.0	2.81	50.9	8.36	116.5		
	3	0.00	0.0	1.10	19.9	8.93	124.5		
	5	0.00	0.0	1.00	18.1	8.08	112.7		
	7	0.00	0.0	0.00	0.0	7.00	97.6		
	10	0.00	0.0	0.00	0.0	5.06	70.5		
	15	0.00	0.0	0.00	0.0	5.56	77.5		
	20	0.00	0.0	0.00	0.0	8.10	112.9		
4**	Control	1.75	100.0	4.07	100.0	8.11	100.0	5.22	100.0
(66)	0.05	0.97	55.4	3.32	81.5	7.24	89.2	5.35	102.5
	0.25	0.00	0.0	3.75	92.1	7.86	96.9	5.48	104.9
	0.5	0.00	0.0	2.67	65.6	7.85	96.8	5.07	97.1
	1	0.00	0.0	2.40	58.9	8.56	105.3	5.58	106.8
	3	0.00	0.0	1.16	28.5	8.45	104.2	6.22	119.1
	5	0.00	0.0	0.29	7.1	7.79	96.0	6.23	119.3
	7	0.00	0.0	0.00	0.0	7.30	90.0	5.44	104.2
	10	0.00	0.0	0.00	0.0	7.13	87.9	5.47	104.8
	15	0.00	0.0	0.00	0.0	7.33	90.3	6.06	116.0
	20	0.00	0.0	0.00	0.0	6.01	74.1	6.11	117.0
5	Control	1.46	100.0	5.75	100.0	6.83	100.0	5.52	100.0
(66)	0.05	0.79	54.1	4.85	84.3	7.31	107.0	6.35	115.0
	0.25	0.27	18.4	3.80	66.0	8.14	119.1	6.62	119.9
	0.5	0.00	0.0	3.30	57.3	7.80	114.2	6.35	115.0
	1	0.00	0.0	1.77	30.7	7.80	114.2	6.66	120.7
	3	0.00	0.0	0.42	7.3	8.35	122.2	7.50	135.9
	5	0.00	0.0	0.57	9.9	7.98	116.8	7.42	134.4
	7	0.00	0.0	0.32	5.5	6.99	102.3	7.02	127.1
	10	0.00	0.0	0.00	0.0	4.52	66.1	6.68	121.0
	15	0.00	0.0	0.00	0.0	3.83	56.0	7.61	137.8
	20	0.00	0.0	0.00	0.0	3.02	44.2	7.44	134.8
6	Control	1.15	100.0	5.91	100.0	7.62	100.0	7.54	100.0
(65)	0.05	0.90	78.2	4.50	76.0	6.61	86.7	5.87	77.8
	0.25	0.20	17.4	4.18	70.7	7.50	98.4	6.92	91.7
	0.5	0.00	0.0	3.42	57.8	7.68	100.8	6.28	83.3
	1	0.00	0.0	1.53	25.8	7.13	93.5	5.60	74.2
	3	0.00	0.0	0.85	14.3	7.99	104.8	6.91	91.6
	5	0.00	0.0	0.36	6.0	7.51	98.5	8.00	106.1
	7	0.00	0.0	0.00	0.0	6.22	81.6	7.55	100.1
	10	0.00	0.0	0.00	0.0	6.13	80.4	7.74	102.6
	15	0.00	0.0	0.00	0.0	4 81	63.1	7.94	105.3
	20	0.00	0.0	0.00	0.0	4.17	54.7	7.00	92.8

* Numbers in parentheses indicate number of colonies used.

** Colonies measured after 8, 24, 53 and 72 hours.

used. This was made with lactose and Duggar's modification of Richard's solution. Twenty-five cc. of M/2 lactose, 10 cc. of M/1 KNO_3, 10 cc. of M/4 KH_2PO_4, 5 cc. of M/10 $MgSO_4$ and a trace of iron were substituted for the potato and dextrose in the usual potato-dextrose agar. Otherwise the medium was prepared as before.

All cultures were allowed to grow for 24 hours before irradiation with ultra-violet. In some experiments the cultures were placed in an incubator at 30°C., in some they were grown at the temperature of the laboratory (about 25°C.) and in other experiments they were grown at 21°C. During this period the cultures became adjusted to the agar medium and a normal growth rate was established.

Just before irradiation the colonies were measured. Two lines were drawn at approximately right angles to each other on the bottom of the Petri dish, intersecting at the center of each colony. Measurements were made with the aid of a binocular dissecting microscope set on a ground glass plate illuminated from below. A piece of millimeter graph paper was placed on the plate to serve as a scale. The covers were removed from the dishes to facilitate measuring. By focusing the microscope on the graph paper and the colony, measurements could be made which were accurate to within 1 to 2 tenths of a millimeter. The widths of each colony were measured along the two lines.

A Cooper-Hewitt mercury arc lamp operating at 7.5 amperes and 110 volts served as a source of ultra-violet radiation. The lamp was allowed to run for at least 30 minutes before each experiment so that the intensity of the radiation was fairly constant. The full spectrum of the lamp was employed. In this paper unless it is otherwise stated reference to ultra-violet so far as it is used experimentally includes some infra-red and some visible radiation. This liberty is taken with the assumption that biological effects are due, as the extensive literature seems to indicate, to energy in the ultra-violet exclusively or practically so.

The uncovered cultures were given different exposures to radiation in a constant temperature bath at 0°C. The distance between the lamp and the culture was 40 cm. except in one set of experiments in which this distance was increased to 150 cm. After irradiation the cultures were allowed to grow either at the temperature of the laboratory (about 25°C.), at 30°C. or at 21°C. At intervals the colonies were measured along the same lines as before.

The principal sources of error in this method are the limitations on the number of individuals which can be measured in a single experiment and the presence of by-products in the medium which affect the growth rate of older cultures.

Data and results

Table 1 gives the comparative rates of growth of irradiated and control cultures during various intervals of time following irradiation. The same results are represented graphically in figure 1. For these data the colonies were grown at the temperature of the laboratory (about 25°C.) before and after irradiation. Stimulation of the growth rate did not occur except after a previous retardation of growth, and the amount of stimulation in any one experiment is roughly proportional within certain limits to the amount of previous retardation. This is shown by the growth averages in table 1. This

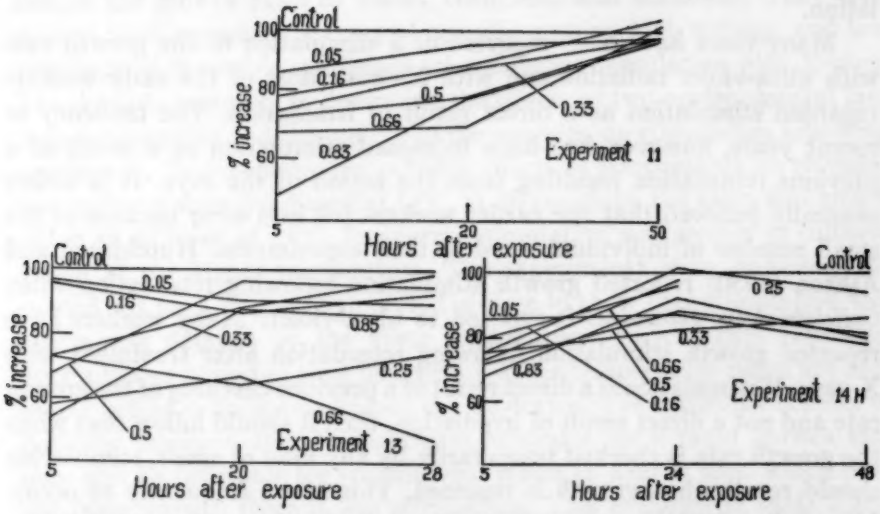

Fig. 2. The lack of initial stimulation with short exposures to radiation as shown by the comparative growth rates of irradiated and control cultures during various intervals of time following irradiation. The period of irradiation in minutes is indicated on each curve. The cultures were irradiated at 150 cm. instead of at 40 cm. as in the other figures.

table also shows that with every exposure to radiation the total increase in diameter is less than in the controls. In general the maximum amount of stimulation occurs during the 24–48 hour interval after exposures of 3 or 5 minutes. The initial retardation bears a direct relation to the length of the exposure. The growth obtained with 15 and 20 minute exposures is quite variable so that averages for these long exposures are not so significant. After 72 hours growth becomes quite variable owing perhaps to the accumulation of harmful by-products in the medium. Also the averages obtained for 72 hours are usually based on a smaller number of measurements. By this time the colonies had frequently become so crowded that accurate measurements could not be made.

The fact has been emphasized that stimulation of the growth rate does not occur except after a previous retardation. Frequent reports in the literature of an initial stimulation with small doses of ultra-violet radiation led to the experiments recorded graphically in figure 2. For these experiments the distance between the lamp and the culture was increased from 40 to 150 cm. The amount of energy from a 40-second exposure at 150 cm. is roughly equivalent to that from a 3-second exposure at 40 cm., and that from a 3-second exposure at 150 cm. is roughly equivalent to that from 3/14 of a second at 40 cm. In no case was there any initial stimulation.

Many cases have been reported of a stimulation of the growth rate with ultra-violet radiation and with X-rays. Most of the early workers regarded stimulation as a direct result of irradiation. The tendency in recent years, however, has been to regard stimulation as a result of a previous retardation resulting from the action of the rays. It is rather generally believed that the earlier workers fell into error because of the small number of individuals used in their experiments. Hutchinson and Ashton (1930) reported growth stimulation following retardation when *Colletotrichum phomoides* is exposed to ultra-violet. Many workers have reported growth stimulation following retardation after treatment with X-rays. If stimulation is a direct result of a previous checking of the growth rate and not a direct result of irradiation, then it should follow that when the growth rate is checked temporarily by any kind of agent, stimulation should result when growth is resumed. This seems frequently to occur. Miss Colley (1931) reported that cells of *Escherichia coli* are initially inhibited when grown in 0.0001 N and 0.00025 N $ZnSO_4$, but after 16 to 20 hours they increased in number so that after 24 hours the colonies were 9 per cent larger than the controls. In other bacteria, however, she observed an initial stimulation. Townsend (1897) reported retardation and a subsequent stimulation of growth of barley and corn seedlings following mechanical injuries. However, he reported also an initial acceleration with very slight injuries. Experiments were made by the author with low temperatures on *Fusarium Eumartii*. An exposure of 5 hours to 0°C. or 45 minutes to −7.5°C. produced a marked retardation followed by stimulation. Shorter exposures or higher temperatures, or both, never produced an initial stimulation. These results are in agreement with those obtained with ultra-violet radiation. They support the idea that stimulation is not due to something inherent in radiation alone but to something characteristic of a number of factors whose only common property is their ability to inhibit growth. This does not preclude the possibility of an initial stimu-

lation. Its existence, however, will be questioned until there has been adequate verification with quantitative methods.

The amount of stimulation recorded in table 1 varies markedly in different experiments. It was considered that certain environmental conditions might affect the amount of stimulation produced. This question was discussed by Hutchinson and Newton (1930) for yeasts. In their experiments with visible light they always obtained the greatest stimulation when the control had a slow rate of growth. On the other hand, Shull and Mitchell (1933) using X-rays reported that harmful effects mask stimulation of the growth rates of wheat, corn, oats and sunflower. They considered brief exposures, the use of metallic screens, high voltage, and low amperage as favorable conditions for producing stimulation with X-rays. I have made attempts to determine the effects of two environmental factors, temperature and the quality of the medium, on stimulation following irradiation. To determine the possible effects of temperature on stimulation cultures were irradiated at O°C. at 40 cm. from the lamp and grown either at 30°C. or at 21°C. In general stimulation was greater in cultures grown at the higher temperature. The experiments were, however, not carried out for a long enough period of time to show that stimulation was not merely delayed by the lower temperature. Experiments extending over a longer period of time and with a wider temperature range are necessary before definite conclusions can be drawn. The experiments seem to indicate, however, that temperatures favorable for a rapid growth rate may also be favorable for stimulation. When an agar is used which does not favor growth there is less stimulation. This is shown in table 2 and figure 3. In experiment 2 a potato-dextrose agar was used which was accidentally partly precipitated. Spores would not germinate on this agar and mycelial growth was very slow. A so-called "poor" agar in which lactose was the source of carbon was used in experiment 10P. The preparation of this agar has already been described. In 10G the usual potato-dextrose agar was used. The cultures were grown at 30°C. in experiments 10G and 10P and at about 25°C. in experiment 2. If results could be obtained for other environmental factors similar to those obtained for temperature and the quality of the medium, then it could be stated that any factor favorable to the growth of the culture is favorable also to stimulation. The results reported here at least suggest this conclusion.

Previous workers have suggested that when growth is retarded labile products may be stored in the plant until conditions are again favorable for growth. It seems possible that growth acceleration results immediately following retardation because of this accumulated supply of labile products. If this is the case it does not seem unreasonable to assume that any

TABLE 2

The mean increase in diameter (in mm. and as % of control) of irradiated and control cultures during various intervals of time following irradiation. Experiments 2 and 10P were made with poor nutrient agars and experiment 10G with a good nutrient agar.

EXP. NO.	MINUTES EXPOSURE	0–24 HRS.		24–48 HRS.		48–72 HRS.	
		AV.	%	AV	%	AV.	%
10G	Control	9.04	100.0	8.78	100.0	8.32	100.0
(45)*	0.05	7.98	88.2	9.22	105.0	9.10	109.3
	0.25	6.79	75.1	9.03	102.8	8.85	106.3
	0.5	5.20	57.5	9.30	105.9	8.77	105.4
	1	4.58	50.6	9.80	111.6	9.47	113.8
	3	2.40	26.5	9.85	112.1	9.33	112.1
	5	2.68	29.6	10.23	116.4	8.93	107.3
	7	0.60	6.6	9.80	111.6	8.16	98.0
	10	0.54	5.9	9.17	104.4	9.20	110.6
	15	0.00	0.0	9.31	106.0	8.85	106.3
10P	Control	7.49	100.0	7.77	100.0	6.91	100.0
(55)	0.05	6.43	85.8	8.23	105.9	7.95	115.0
	0.25	4.59	61.2	7.42	95.5	8.43	121.9
	0.5	2.91	38.8	7.74	99.6	8.24	119.2
	1	0.41	5.4	9.63	123.9	7.93	114.7
	3	0.00	0.0	3.75	48.2	8.68	125.6
	5	0.00	0.0	3.65	46.9	9.32	134.8
	7	0.00	0.0	2.30	29.6	8.33	120.5
	10	0.00	0.0	1.65	21.2	8.70	125.9
	15	0.00	0.0	0.00	0.0	5.60	81.0
2**	Control	0.60	100.0	5.60	100.0	4.30	100.0
(33)	0.05	0.75	125.0	4.46	79.6	4.11	95.5
	0.25	0.17	28.3	3.75	66.9	3.60	83.7
	0.5	0.10	16.6	3.75	66.6	4.23	98.3
	1	0.07	11.6	3.32	59.2	4.17	96.9
	3	0.07	11.6	1.95	34.8	3.85	89.5
	5	0.06	10.0	1.61	28.7	3.65	84.8
	7	0.06	10.0	1.08	19.2	3.28	76.2
	10	0.10	16.6	2.10	37.5	3.81	88.6
	15	0.05	8.3	0.85	15.1	3.22	74.8
	20	0.01	1.6	0.52	9.2	2.52	58.6

* Numbers in parentheses indicate number of colonies used.

** Colonies measured after 5, 31 and 48 hours.

factor which favors the formation of labile products also favors stimulation following retardation. One might also expect that any factor which would increase the growth rate of controls would increase the formation of labile products in exposed cultures. This theory involving labile products is purely hypothetical but it does offer a rather satisfying explanation of the results obtained here and in the investigations reported by others. It does not, however, give any explanation for cases reported of initial

stimulation. The possibility certainly exists that experiments which show initial stimulation were so designed that a retardation might have occurred unobserved. It is difficult, for example, to conceive of a mechanical

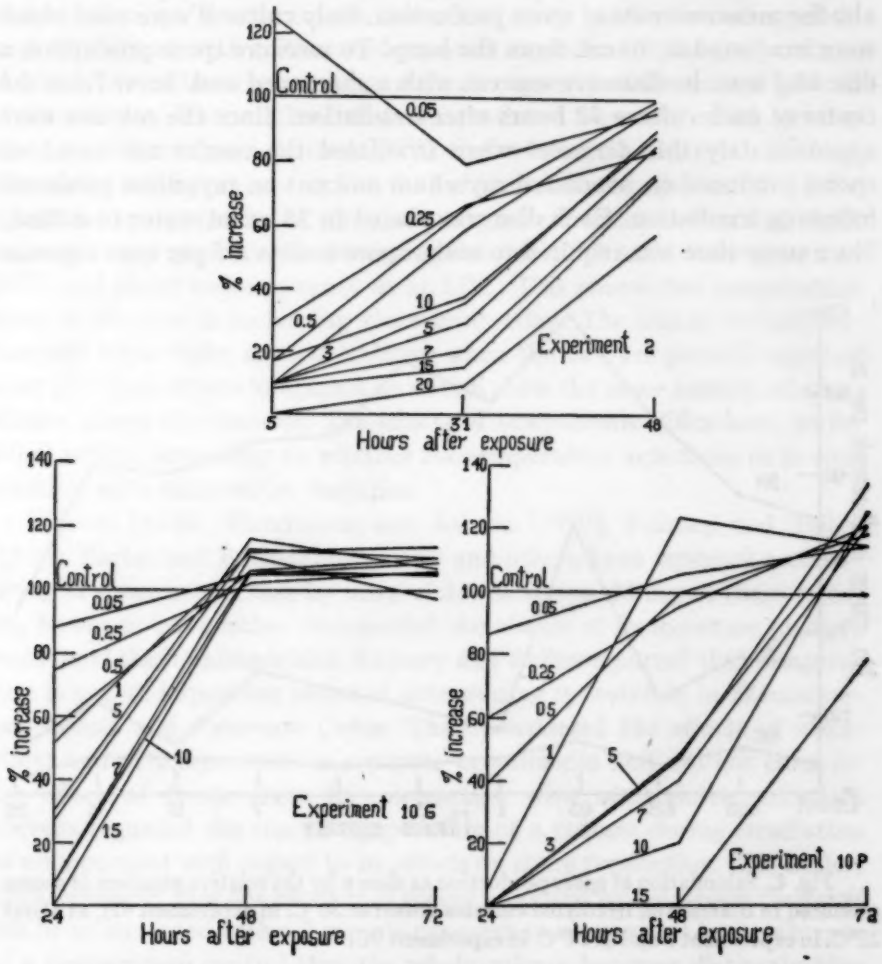

Fig. 3. The depressing effect on stimulation of a poor nutrient medium as shown by comparative growth rates of irradiated and control cultures during various intervals of time following irradiation. The period of irradiation in minutes is indicated on each curve. Experiments 2 and 10P were made with poor nutrient agars and experiment 10G with a good nutrient agar.

injury to a seedling which does not at least temporarily interrupt growth in some part. The mass of the literature demands an hypothesis involving growth retardation.

STIMULATION OF SPORE PRODUCTION

Methods

Some of the cultures used for growth-rate measurements were used also for measurements of spore production. Only cultures were used which were irradiated at 40 cm. from the lamp. To measure spore production a disc 18.5 mm. in diameter was cut with a sharpened cork borer from the center of each culture 72 hours after irradiation. Since the colonies were approximately this diameter when irradiated the results are based on spores produced on irradiated mycelium and not on mycelium produced following irradiation. Each disc was placed in 25 cc. of water in a flask. Since some time was required to make spore counts a 5 per cent aqueous

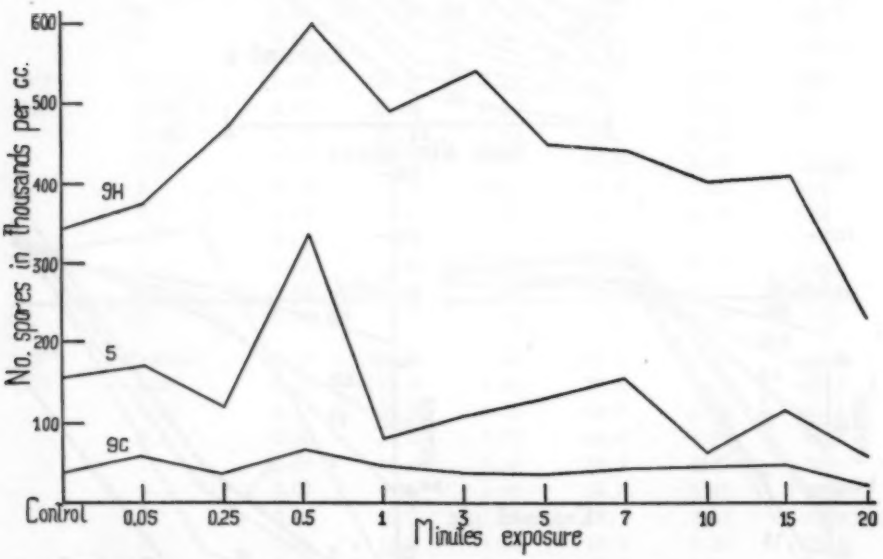

Fig. 4. Stimulation of spore production as shown by the relative numbers of spores produced in control and irradiated cultures grown at 30°C. in experiment 9H, at about 25°C. in experiment 5 and at 21°C. in experiment 9C.

solution of formalin instead of distilled water was sometimes used to prevent spore germination. The number of spores per cubic centimeter of spore suspension was determined with a Levy counting chamber. It is very important that the suspension be thoroughly shaken to dislodge all the spores from the mycelium before counts are made. To insure this the suspension was thoroughly shaken before each sample was taken. In each case samples were drawn until there was fairly close agreement between consecutive counts.

Data and results

Table 3 and figure 4 show the number of spores per cubic centimeter of suspension produced in cultures exposed to various amounts of ultra-violet radiation. The maximum amount of spore production occurred in each experiment with a 30-second exposure regardless of the temperature at which the cultures were grown and consequently of the rate of growth of the culture. The number of spores produced was very much greater when the cultures were grown at 30°C. than when grown at 21°C. With an intermediate temperature such as that of the laboratory (about 25°C.) the number of spores produced was also intermediate. There were about eight times as many spores in the controls grown at 30°C. as in those grown at 21°C. and about twice as many as at 25°C. This shows that temperature alone is effective in increasing spore production. The effects of temperature and ultra-violet are not additive when the two are present together since the three curves in figure 4 do not all show the same amount of stimulation above the controls. The effects of temperature differ here, as for lethal action, depending on whether the temperature acts alone or in conjunction with ultra-violet radiation.

Stevens (1930), Hutchinson and Ashton (1930), Ramsey and Bailey (1930), Porter and Bockstahler (1928) and others have reported a stimulation of spore production by ultra-violet radiation. Many of these workers, however, have either disregarded the effects of temperature or have considered them unimportant. Ramsey and Bailey reported that temperature is not an important factor in determining sporulation in *Macrosporium tomato* and *Fusarium Cepae*. They considered the effects of ultra-violet and of temperature in separate experiments and did not consider the effect of temperature in conjunction with ultra-violet radiation. Stevens regarded the rise in temperature of a culture during irradiation as unimportant with regard to its effects on spore production. The author finds, however, that the number of spores produced by *Fusarium Eumartii* can be so increased with a 2-minute exposure to ultra-violet in the absence of a temperature control that the whole culture becomes distinctly blue in color. The blue color is due to diffraction by a large number of spores and not to pigmentation. The blue color was not obtained when the cultures were irradiated in ice water or in a water bath at room temperature. With this fungus, at least, an accurate measure of spore production under the influence of ultra-violet radiation cannot be obtained without temperature control.

It is rather generally considered that spores are produced only when there is a retardation of vegetative growth. This raises the question as to

TABLE 3

The effects of ultra-violet on spore production in cultures grown at different temperatures.

EXP. NO.	TEMPERATURE °C.	MINUTES EXPOSURE	AV. NO. SPORES/CC. OF SUSPENSION
5	25	Control	156,700
(39)*		0.05	171,000
		0.25	120,000
		0.5	336,000
		1	78,500
		3	112,700
		5	128,500
		7	155,000
		10	68,700
		15	116,500
		20	59,200
9H	30	Control	336,200
(56)		0.05	374,100
		0.25	476,100
		0.5	602,100
		1	484,000
		3	542,000
		5	452,600
		7	442,700
		10	397,000
		15	405,000
		20	230,300
9C	21	Control	39,700
(61)		0.05	61,800
		0.25	40,400
		0.5	62,500
		1	50,800
		3	44,600
		5	45,800
		7	44,500
		10	48,000
		15	57,500
		20	22,800

* Numbers in parentheses indicate number of colonies used.

whether the stimulation of spore production with ultra-violet radiation arises from a retardation of the growth rate following irradiation. A study of growth rates with a 30-second exposure, however, reveals nothing which might explain a maximum spore production with this exposure. Also many factors which affect the growth rate apparently do not influence spore production. These facts do not prove but they do indicate that it is very likely that ultra-violet acts directly here as a stimulant to spore produc-

tion. If this is so, the manner of stimulation of spore production forms an interesting contrast to that of stimulation of the growth rate.

SUMMARY

1. Stimulation of vegetative growth was never obtained without a previous retardation, and even when stimulation occurred the total growth was never greater than in the controls. There were no cases of an initial stimulation. Stimulation is considered as merely an indirect effect of radiation and a direct effect of retardation since other agents which produce a retardation also produce stimulation. Temperature conditions and nutritional conditions which favor the growth of the fungus favor also stimulation. The idea is suggested that those conditions which favor the formation and accumulation of labile products favor also stimulation following retardation.

2. The maximum stimulation to spore production occurs with a 30-second exposure to ultra-violet radiation regardless of the growth rate of the fungus. Temperature alone is effective in stimulating spore production. Since the amount of stimulation with ultra-violet is different in cultures grown at different temperatures the effects of ultra-violet and temperature are not additive. Since a study of growth rates reveals nothing which might explain a maximum spore production with a 30-second exposure to ultra-violet and since many other factors which influence the growth rate have not been found to influence spore production, ultra-violet may act directly rather than indirectly to stimulate spore production.

This investigation was proposed by Prof. B. M. Duggar and has been carried on with his suggestions and criticisms. The author is indebted to Prof. Duggar and to Prof. H. H. Bartlett for assistance in the preparation of the manuscript. The latter part of this investigation was carried on at the University of Michigan with the aid of an F. C. and Susan Eastman Newcombe Fellowship.

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INDEX TO AMERICAN BOTANICAL LITERATURE

1931–1934

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